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(54) **Title**: JAK INHIBITORS FOR ACTIVATION OF EPIDERMAL STEM CELL POPULATIONS

(57) **Abstract**: The invention provides methods to activate epidermal stem cells and/or progenitor cells by interfering with the JAK/STAT signalling pathway. More specifically, JAK inhibitors are provided for use in epidermal stem cell activation and cosmetic or pharmaceutical applications derived thereof, such as promoting hair growth, treatment of hair-loss disorder, repair and regeneration of the skin.

JAK INHIBITORS FOR ACTIVATION OF EPIDERMAL STEM CELL POPULATIONS

FIELD OF THE INVENTION

The present invention relates to the fields of aging and age-associated changes in stem cells. In particular, the invention provides methods to activate epidermal stem cells and/or progenitor cells by interfering with the JAK/STAT signalling pathway. More specifically, JAK inhibitors are provided for use in epidermal stem cell activation and cosmetic or pharmaceutical applications derived thereof, such as promoting hair growth, treatment of hair-loss disorder, repair and regeneration of the skin.

BACKGROUND

Adult tissue homeostasis requires continual replacement of cells lost due to normal turnover, injury, and disease. However, aging is accompanied by decreased tissue regeneration and homeostasis, both of which are frequently associated with impaired stem cell function. As some stem cell compartments undergo dramatic age-associated changes, including alterations in cell number, decreased regenerative capacity and fate-change, a decline in stem cell function is suggested to contribute to the aging process (Conboy et al. 2003; Rossi et al. 2005; Molofsky et al. 2006). Therefore, characterizing the regulatory mechanisms that mediate normal stem cell aging is critical to understanding age-associated pathologies and disease, and to devising mechanisms for treatment.

The skin is one of the most obvious tissues to undergo aging-associated phenotypic and functional changes, including decreased hair cycling, epidermal thinning, diminished sebaceous gland function and an impaired wound response (Balin and Pratt 1989). The cellular regeneration of the skin is maintained by different adult stem/progenitor cell subpopulations localized within the specialized microenvironments, niches in interfollicular epidermis (IFE), sebaceous gland and hair follicle bulge region (Mimeault and Batra 2010). Although some studies in mice suggest that epidermal stem cells are retained during aging (Stern and Bickenbach 2007; Giangreco et al. 2008), aged human keratinocyte stem cells exhibit decreased colony-forming ability (Barrandon and Green 1987), suggesting that undiscovered stem cell changes may be involved in epidermal aging.

Stem cell therapy is one of the current strategies to promote tissue growth in vivo or to generate cultured tissues for transplantation (reviewed by Vogel 1999). Methods for skin regeneration using mesenchymal stem cells are, for example, disclosed in EP0953040. However, in addition to ethical concerns, the use of extrinsic stem cells raises concerns regarding tumorigenicity caused by undifferentiated pluripotent cells as well as immunogenicity caused by allogenecity.

Thus, there is a need for agents capable of maintaining, increasing and/or activating the pool of endogenous (pre-existing) epidermal stem cells and/or progenitor cells, thus avoiding the many drawbacks involved in current stem cell therapies, in order to promote epidermal renewal and prevent and/or combat cutaneous signs of aging.

SUMMARY OF THE INVENTION

Altered stem cell homeostasis is linked to organismal aging. However, the mechanisms involved remain poorly understood. The inventors have discovered that hair follicle stem cells are susceptible to age-associated changes that may contribute to aging phenotypes. Novel unexpected alterations in hair follicle stem cells during skin aging were identified, including increased numbers, decreased function and an inability to tolerate stress. That these cells retain stem-like properties while simultaneously being repressed by the external environment implies a degree of reversibility, which the inventors show is feasible with chemical compounds.

The inventors found that inhibitors of the JAK/STAT signalling pathway can be used to activate a population of epidermal stem cells and/or progenitor cells. Advantageously, this may lead to several applications in the cosmetics and/or pharmaceutical field, including but not limited to regeneration and repair of skin, promotion of hair growth and treatment of hair-loss disorders. Avoiding the drawbacks associated with conventional stem cell therapy procedures such as stem cell isolation, preparation, surgery, extraction, etc., the present invention thus provides methods for activation of adult epidermal stem cells *in situ* to undergo differentiation into the many cell types required to generate tissue, by localized delivery of inhibitors of the JAK/STAT signalling pathway.

It is thus an object of the invention to provide a JAK inhibitor for use in activation of a population of epidermal stem cells and/or progenitor cells. In one particular embodiment, the population of epidermal stem cells and/or progenitor cells is a population of hair follicle stem cells and/or progenitor cells. Preferably, the population of epidermal stem cells and/or progenitor cells is a human stem cell population. In particular, the JAK inhibitor is an inhibitory agent or a small molecule. Preferably, the JAK inhibitor for use according to the invention is an inhibitor of JAK3.

Within the scope of the present invention, several applications are envisaged including (i) the JAK inhibitor for use in hair follicle regeneration, (ii) the JAK inhibitor for stimulating hair growth and/or for use in treatment of a hair-loss disorder; (iii) the JAK inhibitor for use in skin repair and regeneration; (iv) the JAK inhibitor for use in wound healing.

Typically, the JAK inhibitor for use according to the invention will be formulated in a physiologically acceptable medium. The cosmetic and pharmaceutical compositions utilized in this invention may be administered by any number of routes including oral, transdermal, subcutaneous and topical. Preferably, the JAK inhibitors for use according to the invention will be formulated in a physiologically acceptable medium that is suitable for topical application.

Further, the invention relates to a method for activating a population of epidermal stem cells and/or progenitor cells, the method comprising contacting epidermal tissue or cells with an effective amount of a JAK inhibitor, in particular a JAK inhibitor that inhibits JAK3.

Also provided is a method for stimulating hair growth and/or treating a hair-loss disorder in a subject in need thereof, the method comprising applying to a subject in need thereof an effective amount of a JAK inhibitor, in particular a JAK3 inhibitor, formulated in a physiological acceptable medium.

The invention further envisages a method for skin regeneration or for wound healing, the method comprising applying to a site in need thereof an effective amount of a JAK inhibitor, in particular a JAK3 inhibitor, formulated in a physiological acceptable medium.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Krt-15-GFP hair follicle stem cell number increases with age.

a, Whole-mount co-immunofluorescence of aged tail epidermis stained with antibodies targeting Keratin 15 (red) and GFP (green). **b,c,** Quantification of absolute GFP cell number per hair follicle **(b)** and fluorescence intensity relative to Krt-15 protein (n=32-42 follicles from 3 mice) **(c)**. **d,** Percentage of Krt-15-GFP^{+} cells in epidermal preparations analysed by FACS (data points represent independent FACS analyses of 1-3 mice; n≥7 mice total per time point). **e,** Whole-mount immunofluorescence for Krt-15 protein in aged tail epidermis and intensity quantification relative to DAPI. **f,** Representative FACS scatter plots and summarized percentages of CD34^{+}/CD49f^{hi} cells in aged epidermis (n=4-5). **g,** FACS analysis of aging GFP^{+}/CD34^{+}/CD49f^{+} cells (n=3). Scale bar=100um. p-values were determined using unpaired, two-tailed student t-tests. *p<0.05, **p<0.005. n.s.=not significant.

Figure 2. Age-associated functional decline in Krt-15-GFP stem cells.

a, Clonogenic colony forming assays of FACS-sorted GFP^{+} and GFP^{-} cells from 3- and 18-month old epidermis. Pictured are duplicate wells of each condition, representative of n≥3 independent experiments. **b,c,** Quantification of colony number **(b)** and normalized colony size **(c)** of data depicted

in **(a)**. **d,** Colony assays using FACS-sorted $GFP^+/CD34^+/CD49f^+$ cells. **e,** Aged Krt-15-GFP mice were exposed to 5Gy whole-body irradiation and sacrificed 24h post IR. Shown is a graph of the FACS-determined fold change in GFP^+ cells in IR-treated epidermis relative to non-IR controls at each timepoint. **f,** Absolute GFP^+ cell number per hair follicle in shaved, back skin epidermis treated with 20nM TPA every other day for one week. **g,** Representative histological images of back skin sections stained with antibodies against Ki67, GFP and CD34. p-values were determined using unpaired, two-tailed student t-tests. *p<0.05, **p<0.001. Error bars for bar graphs represent +/- s.d.

Figure 3. Heatmaps of epidermal, lineage-associated transcripts (3m vs. 18m).

a-c, Heat maps depicting relative gene expression changes for transcripts associated with interfollicular epidermis **(a)**, core hair follicle bulge **(b)**, and sebaceous gland **(c)**. Individual transcript values for each time point (row) are shown as the log2(fold change [time point]) with respect to the mean expression value.

Figure 4. Selected qPCR of epidermal stem cell transcripts (3m vs. 18m).

Quantitative RT-PCR of selected epidermal stem cell transcripts. n=3 RNA pools (n=1-3 mice/pool) for each time point. p-values were determined using unpaired, two-tailed student's t-tests. *p<0.05.

Figure 5. Dynamic changes in cytokine signaling networks in aging epidermis.

a, A balloon graph depicting RNA-seq-determined individual transcript changes of aged, Krt-15-GFP stem cells. Red data points represent significantly up or down regulated transcripts. **b,** Differential expression values of transcripts associated with positive (+) or negative regulation (-) of selected signalling pathways. **c,** qRT-PCR validation of selected Jak-Stat signalling transcripts. Data represents n=3 independently FACS-sorted RNA pools (mice) for each time point. p-values were determined using unpaired, two-tailed student t-tests. *p<0.05, **p<0.005. n.s.=not significant. Error bars for bar graphs represent +/- s.d.

Figure 6. Gene ontology analysis of significant differentially expressed transcripts.

a,b, Gene ontology analysis of significant differentially expressed genes between 3- and 18- month Krt-15-GFP cells. Transcripts were analysed using Gene Ontology enrichment analysis and visualization (GOrilla) software (http://cbl-gorilla.cs.technion.ac.il/). Shown are the top 20 GO processes enriched in up-regulated **(a)** and down-regulated **(b)** gene sets.

Figure 7. Jak-kinase inhibition promotes aged epidermal stem cell function *in vitro* and *in vivo*.

a, Clonogenic colony formation assays of aged (18m) keratinocytes cultured in the presence of Jak, Wnt, or Notch pathway inhibitors. **b,** Colony assays of FACS-sorted GFP^+ or $GFP^+/CD34^+/CD49f^+$ cells treated with 500nM Jak inhibitor. **c,** Representative whole-mount immunofluorescence images of Jak-inhibitor treated, aged tail epidermis stained with antibodies targeting Ki67 (left, red) and CDP (right, green). Scale bar=200um. **d,** Quantification of anagen hair follicles in Jak-inhibitor treated tail hair follicles compared to vehicle controls. Data is representative of three independent *in vivo* experiments (n=5 mice). **e,** Representative histological images of Jak-inhibitor treated back skin epidermis stained using antibodies targeting GFP, CD34 or Ki67. p-values were determined using unpaired, two-tailed student t-tests. *p<0.05, **p<0.0001. Error bars for bar graphs represent +/- s.d.

Figure 8. Colony immunofluorescence (JAK inhibitor), colony assays: additional pathway modifying drugs.

a, Representative histological and immunofluorescent images of aged keratinocytes treated with vehicle or 500nM Jak inhibitor (Jaki). Scale bar=50um. **b,** Clonogenic colony forming assays of drug-treated 18-month old epidermis. Epidermal cell preparations were generated from wild type C57Bl6/J mice, allowed to attach to a fibroblast feeder layer, and then treated with the Wnt-agonist BIO (500nM-1uM) or Cyclopamine (1uM-5uM).

Figure 9. Additional JAK inhibitors, serial enrichment colony assays.

Clonogenic colony forming assays using 18m epidermis cultured in the presence of either vehicle (left column)), or the Jak-Stat inhibitors Ruxolitinib (bottom row) or Tofacitinib (top row).

Figure 10. Sorted CD34/GFP subpopulation colony assays +/- IR.

a, Clonogenic colony forming assays of FACS-purified progenitor cell subpopulations. Aged (3m and 18m) CD49f+-gated cells were sorted, split into 3 groups (bulge+/GFP+, bulge+/GFP-, and bulge-/GFP+), and treated +/- 500nM Jak inhibitor. **b,** Colony assays (as described in [a]) instead using tissue sourced from mice exposed to 5Gy whole-body irradiation for 24h.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will be described with respect to particular embodiments and with reference to certain drawings but the invention is not limited thereto but only by the claims. Any reference signs in the claims shall not be construed as limiting the scope. The drawings described are only schematic and

are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes. Where the term "comprising" is used in the present description and claims, it does not exclude other elements or steps. Where an indefinite or definite article is used when referring to a singular noun e.g. "a" or "an", "the", this includes a plural of that noun unless something else is specifically stated. Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

Unless otherwise defined herein, scientific and technical terms and phrases used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclatures used in connection with, and techniques of molecular and cellular biology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, for example, Sambrook et al. Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992, and Supplements to 2002); Leach, Molecular Modelling: Principles and Applications, 2d ed., Prentice Hall, New Jersey (2001).

Definitions

As used herein, a “stem cell” is a cell that can divide and differentiate into diverse specialized cell types and that can self-renew. Generally, stem cells can divide without limit and are totipotent. The role of stem cells *in vivo* is to replace cells that are destroyed during the normal life of a multicellular organism. After division, the stem cell may remain a stem cell, become a precursor cell, or proceed to terminal differentiation. In mammals, there are two main types of stem cells: embryonic stem cells found in blastocysts and adult stem cells found in various tissues. The stem cells as referred to in the present invention are meant to be adult stem cells. As used herein, “adult stem cells” are cells that have the ability to self-renew throughout adult life and to generate progeny that undergo further differentiation. In the present invention, adult stem cells found in epidermal tissue are particularly envisaged, and will be described further herein.

As used herein, a "progenitor cell" is a cell that, like a stem cell, has the capacity to differentiate into a specific type of cell, but is already more specific than a stem cell and is pushed to differentiate into its target cell. The most important difference between stem cells and progenitor cells is that stem cells can replicate indefinitely, whereas progenitor cells can divide only a limited number of times.

"JAK" or Janus Activated Kinases are cytoplasmic tyrosine kinases that are either constitutively associated with cytokine receptors or recruited to receptors after ligand binding. In either case, stimulation with the ligand results in the catalytic activation of receptor-associated JAKs. This activation results in the phosphorylation of cellular substrates, including the JAK-associated cytokine receptor chains. Some of these phosphorylated tyrosines can serve as coding sites for "STAT" proteins or Signal Transducer and Activator of Transcription proteins, which bind to the phosphotyrosines by their SRC-homology 2 (SH2) domains. STAT proteins are also phosphorylated on a conserved tyrosine residue, resulting in their dimerization and acquisition of high-affinity DNA-binding activity, which facilitates their action as nuclear transcription factors. The JAK/STAT pathway is one of the most rapid cytoplasmic to nuclear signalling mechanisms. There are a total of four JAK (JAK1-3 and tyrosine kinase 2) and seven STAT proteins (STAT 1-4, STAT5a, STAT5b and STAT6) (for review see Rawlings et al. 2004).

As used herein, the term "skin appendages" in particular means the hair follicles, the sebaceous glands and the nails.

The term "Alopecia Areata" (AA) is known in the art and refers to an autoimmune disease leading to hair loss due to the collapse of the immune privilege of the hair follicle and subsequent autoimmune destruction. AA is a skin disease which leads to hair loss on the scalp and elsewhere. In some severe cases, it can progress to complete loss of the hair on the head or body. The term "androgenetic alopecia" is known in the art and refers to an inherited condition, caused by a genetically determined sensitivity to the effects of dihydrotestosterone, which is believed to shorten the anagen phase of the hair cycle, causing miniaturisation of the follicles, and producing progressively finer hairs. Generally, alopecia can also be induced by chemical agents or physical agents (e.g. during anti-cancer chemotherapy), and the condition also results from specific disease states and factors (emotional distress). Alopecia typically is attributable to a disturbance in the hair renewal cycle leading to acceleration of the frequency of the cycles, which results in a shift in the population of follicles from the anagen phase to telogen. Ultimately, the hair follicles degenerate and a decrease in the number of hairs in the affected area of the scalp or skin is observed.

Detailed description

It is an object of the invention to provide a JAK inhibitor for use in activation of a population of epidermal stem cells and/or progenitor cells. In particular, the population of epidermal stem cells and/or progenitor cells is from mammalian origin, preferably human origin.

Epidermal stem cells and its progenitor cells are localized in the skin epidermis, which forms one of the two tissues of the skin (the other being the dermis, which gives the epidermis a solid support). The skin epidermis is the stratified epithelium that forms a barrier that protects animals from dehydration, mechanical stress, and infections. The epidermis encompasses different appendages, such as the hair follicle (HF), the sebaceous gland (SG), the sweat gland, and the touch dome, that are essential for thermoregulation, sensing the environment, and influencing social behaviour. The epidermis undergoes a constant turnover and distinct epidermal stem cells (SCs) are responsible for the homeostasis of the different epidermal compartments.

The term "population of epidermal stem cells and/or progenitor cells", as used herein, refers to one or more of the multiple populations of adult stem cells and/or progenitor cells (as defined herein) residing in different locations within epidermal tissue and are well-characterized. Under normal homeostatic conditions the stem cells in different locations maintain the differentiated lineages that are appropriate for those locations. Thus, an epidermal stem cell population includes hair follicle stem cells that will maintain the hair lineages, sebaceous gland stem cells that will produce differentiated sebocytes and stem cells in the interfollicular epidermis (IFE) that will give rise to the outermost barrier layers of the epidermis. However, in response to injury or genetic manipulation, different epidermal stem cell populations are functionally interconvertible. Thus, epidermal stem cells are multipotent and may give rise to all cell types of the hair, the epidermis and the sebaceous gland. For example, stem cells in the interfollicular epidermis can be reprogrammed to become hair follicle stem cells on sustained activation of the WNT pathway. Thus, a population of epidermal stem cells as used herein includes such reprogrammed epidermal stem cells. Typically, a population of epidermal stem cells and/or progenitor cells is characterized by the expression of specific markers, preferably molecular markers that distinguish them from other cell types in the epidermal tissue and allow their isolation. Molecular markers of epidermal stem cells are known by the person skilled in the art and non-limiting examples are provided in Morris et al. 2004, Tumbar et al. 2004, Ito et al. 2005.

According to the invention, the expression "activation of a population of epidermal stem cells and/or progenitor cells", as used herein, means, in particular maintaining, promoting and/or increasing (i) the capacity of said population of cells for self-renewal and/or their capacity to proliferate, and/or (ii) their

capacity to regenerate a stratified epidermis and/or all or some of the skin appendages (sebaceous glands, hair follicle, nails, etc.). Measurable parameters and/or evaluation criteria for the maintaining, promoting and/or increasing of the properties of a population of epidermal stem cells can be expressed both in a quantitative and/or qualitative way, and are known by the person skilled in the art, for example, as described in Barrandon and Green 1987, Morris et al. 2004.

The expression "capacity of a cell for self-renewal", as used herein, means a cell capable of dividing so as to give two daughter cells, at least one of which is identical to the mother cell. On the scale of a complex cell population, the notion of self-renewal implies the maintenance of a compartment of cells having phenotypic and functional characteristics that are constant in the course of the successive cell divisions. According to the invention, this will involve the maintenance of a compartment made up of epidermal stem cells and/or epidermal progenitors, in particular characterized by a strong proliferative potential and a capacity to generate a stratified epidermis.

The expression "capacity of a cell to proliferate", as used herein, means a cell capable of multiplying to give two daughter cells, without there being necessarily transmission of the characteristics and of the potential of the mother cell to at least one of the two daughter cells. The proliferation, which may or may not be associated with the self-renewal phenomenon, is liable to result in the gradual decrease or the disappearance of the cellular compartment of interest within the cell population that multiplies. According to the invention, the cell proliferation can be accompanied by a gradual decrease in and/or by the disappearance of the compartment of epidermal stem cells and/or epidermal progenitors, in particular characterized by a strong proliferative potential and a capacity to generate a stratified epidermis.

One of the locations of epidermal stem cells is one or more particular region of the hair follicle. In a particular embodiment of this object of the invention, a JAK inhibitor is provided for use in activation of a population of hair follicle stem cells and/or progenitor cells.

As used herein, a “population of hair follicle stem cells and/or progenitor cells” refers to a population of epidermal stem cells and/or progenitor cells that primarily resides in the bulge of the hair follicle – the bulge is defined as the attachment site of the arrector pili muscle, but may also reside above the bulge, in the isthmus, as well as in the junctional zone. Hair follicle stem cells are multipotent and are able to give rise to all cell types of the hair, the epidermis and the sebaceous gland. Hair follicle stem cells, like other adult stem cells, are thought to be slow-cycling cells, with a superior clonogenicity and proliferative capacity. The first marker that was used to identify stem cells in the bulge was long-term retention of a DNA label, such as tritiated thymidine or 5-bromodeoxyuridine (BrdU). The first

molecular marker of the bulge to be identified was keratin 15 (K15; also known as KRT15). Several additional markers of the bulge have now been described, including CD34, SOX9, LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5), and GLI1. It will be understood by the person skilled in the art that, although there is substantial overlap in the expression of the different bulge markers, the ultimate fate or function of a given cell type might differ according to the presence of one or more particular markers. Molecular markers of the isthmus include LGR6 and/or PLET1 (placenta-expressed transcript 1; also known as C11ORF34 and MTS24). Molecular markers of the junctional zone include LRIG1 (leucine-rich repeats and immunoglobulin-like domains 1).

Unexpectedly, it was determined that it is possible to activate a population of epidermal stem cells and/or progenitor cells, in particular a population of hair follicle stem cells and/or progenitor cells by administering an inhibitor of the JAK/STAT signalling pathway.

In general, a STAT inhibitor, a JAK inhibitor, and a JAK/STAT inhibitor are used to refer to any agent capable of down-regulating or otherwise decreasing or suppressing the amount and/or activity of JAK-STAT interactions. JAK inhibitors down-regulate the quantity or activity of JAK molecules. STAT inhibitors down-regulate the quantity or activity of STAT molecules. Inhibition of these cellular components can be achieved by a variety of mechanisms known in the art, including, but not limited to binding directly to JAK (for example, a JAK-inhibitor compound binding complex, or substrate mimetic), binding directly to STAT, or inhibiting the expression of the gene, which encodes the cellular components. JAK/STAT inhibitors are well known in the art and are described further herein.

In one embodiment of the object of the present invention, the inhibitor is a JAK inhibitor, such as a JAK1 inhibitor, a JAK2 inhibitor, a JAK3 inhibitor, a TYK2 inhibitor. In another embodiment, the inhibitor is a STAT inhibitor, such as a STAT1 inhibitor, a STAT2 inhibitor. According to specific embodiments, the inhibitor is an antisense RNA that specifically inhibits the expression of the gene that encodes the JAK protein or the STAT protein; or the inhibitor is an siRNA that specifically targets the gene that encodes the JAK protein or the STAT protein; or the inhibitor is an antibody that inhibits the JAK protein or the STAT protein; or the inhibitor is a vaccine that inhibits the JAK protein or the STAT protein. Preferably, the inhibitor is a small molecule compound that inhibits the JAK protein or the STAT protein.

According to a preferred embodiment, the present invention provides for a JAK inhibitor for use in the activation of a population of epidermal stem cells and/or progenitor cells. JAK inhibitors include any small molecule compound, antibody, siRNA or vaccine that inhibits JAK (including JAK1, JAK2, JAK3 and TYK2). In an embodiment, "JAK inhibitor" means any small molecule compound, antibody, siRNA or vaccine that inhibits JAK (including JAK1, JAK2 and JAK3). In another embodiment, "JAK inhibitor"

means any small molecule compound, antibody, siRNA or vaccine that inhibits JAK1. In another embodiment, "JAK inhibitor" means any small molecule compound, antibody, siRNA or vaccine that inhibits JAK2. In another embodiment, "JAK inhibitor" means any small molecule compound, antibody, siRNA or vaccine that inhibits JAK3. In another embodiment, "JAK inhibitor" means any small molecule compound, antibody, siRNA or vaccine that inhibits JAK1/2. In a further embodiment, the term "JAK inhibitor" means any small molecule compound that inhibits JAK (including JAK1, JAK2, JAK3 and TYK2). In an embodiment, "JAK inhibitor" means any small molecule compound that inhibits JAK (including JAK1, JAK2 and JAK3). In another embodiment, "JAK inhibitor" means any small molecule compound that inhibits JAK1. In another embodiment, "JAK inhibitor" means any small molecule compound that inhibits JAK2. In another embodiment, "JAK inhibitor" means any small molecule compound that inhibits JAK3. In another embodiment, "JAK inhibitor" means any small molecule compound that inhibits JAK 1/2.

JAK inhibitors include phenylaminopyrimidine compounds (WO2009/029998), substituted tricyclic heteroaryl compounds (WO2008/079965), cyclopentyl-propanenitrile compounds (WO2008/157208 and WO2008/157207), indazole derivative compounds (WO2008/114812), substituted ammo-thiophene carboxylic acid amide compounds (WO2008/156726), naphthyridine derivative compounds (WO2008/112217), quinoxaline derivative compounds (WO2008/148867), pyrrolopyrimidine derivative compounds (WO2008/119792), purinone and imidazopyridinone derivative compounds (WO2008/060301), 2,4-pyrimidinediamine derivative compounds (WO2008/118823), deazapurine compounds (WO2007/117494) and tricyclic heteroaryl compounds (WO2008/079521).

JAK inhibitors include compounds disclosed in the following publications: US2004/176601, US2004/038992, US2007/135466, US2004/ 102455, WO2009/054941, US2007/134259, US2004/265963, US2008/194603, US2007/207995, US2008/260754, US2006/063756, US2008/261973, US2007/142402, US2005/159385, US2006/293361, US2004/205835, WO2008/148867, US2008/207613, US2008/279867, US2004/09799, US2002/055514, US2003/236244, US2004/097504, US2004/147507, US2004/ 176271, US2006/217379, US2008/092199, US2007/043063, US2008/021013, US2004/ 152625, WO2008/079521, US2009/186815, US2007/203142, WO2008/144011, US2006/270694 and US2001/044442. JAK inhibitors further include compounds disclosed in the following publications: WO2003/011285, WO2007/145957, WO2008/156726, WO2009/035575, WO2009/054941, and WO2009/075830. JAK inhibitors further include compounds disclosed in the following patent applications: US Serial Nos. 61/137475 and 61/134338. Specific JAK inhibitors include Jak2-IA, AG490, Pyridone 6, WP1066, LS104, TG101209, TG101348, CP690,550, CP352,664, INCB18424, WHI-Pl 54, CMP6, SB1518, XLOl 9, CEP-701 , INCB20, AUB-6-96 and AZ960.

According to a particularly preferred embodiment, the present invention envisages a JAK inhibitor that inhibits JAK3 for use in the activation of a population of epidermal stem cells and/or progenitor cells. Pyrimidine derivatives exhibiting JAK3 kinase inhibiting activities are described in WO2008/009458. Pyrimidine compounds in the treatment of conditions in which modulation of the JAK pathway or inhibition of JAK kinases, particularly JAK3 are described in WO2008/118822 and WO2008/118823. Fluoro-substituted pyrimidine compounds as JAK3 inhibitors are described in WO 2010/118986. A particularly preferred example of a JAK3 inhibitor includes CP690,550 (tofacitinib, also called tasocitinib).

The advantage of administering JAK inhibitors for stimulating the regenerative power of the epidermal stem cells and/or progenitor cells capable of generating a stratified epidermis and/or all or some of the skin appendages, in "anti-aging" or "anti-hair loss" cosmetic compositions, and/or for preparing pharmaceutical compositions suited for skin regeneration and/or wound healing, is therefore apparent. The JAK inhibitors as described herein that are capable of activating epidermal stem cells and/or progenitor cells are particularly suited to:

i. Stimulate the renewal of the epidermis and/or of all or some of the skin appendages; in particular the treatment of burns;
ii. Prevent and/or combat cutaneous signs of aging, in particular thinning of the skin and/or loss of firmness, of elasticity and/or of tonicity of the skin, and/or the formation of wrinkles and fine lines; and/or smooth out the microrelief of the skin in order to give it once again a young, smooth and firm appearance;
iii. Revitalize the scalp and/or regenerate growth of the hair and/or of body hair.

It is thus an object of the present invention to provide JAK inhibitors as described herein for use in cosmetic and/or pharmaceutical applications, preferably dermatological applications such as skin repair and regeneration, promoting hair growth, treatment of hair-loss disorders, wound repair, and other skin-related conditions.

According to one embodiment, the JAK inhibitors as described herein can be useful for stimulating hair growth, and/or for the treatment of a hair-loss disorder, thus for retaining hair or reducing hair loss.

Hair growth depends on proliferation of hair follicle matrix cells. It alternates between phases of activity and rest. The anagen phase is a period of growth lasting for two to six years. During this time, the follicle is long and deep, and produces thick, well-pigmented hair. Usually, about 90% of all scalp hairs are in the anagen phase at a given time. This growth phase is followed by the catagen phase for few weeks, which corresponds to the follicle base shrinking. The resting period, or telogen phase, lasts

for two to four months. In this phase, the follicle withers even further. Following the telogen phase, the next anagen phase begins, and old hair is dislodged and falls out to make room for a new hair. Numerous factors affect the hair growth cycle, including heredity, hormonal deficiencies or imbalances, diet, stress, chemotherapy or aging.

The term "hair-loss disorder" refers herein to alopecia such as alopecia areata and androgenetic alopecia as well as hair loss caused by other factors (all as defined herein). The uses of JAK inhibitors as described herein embrace promoting new hair growth, promoting hair growth before, during or after chemotherapy, promoting hair growth in hair transplant patients, preventing, stopping or minimizing hair fall out.

In one specific embodiment, the JAK inhibitors as described herein are particularly useful for hair follicle regeneration. As used herein, the expression "hair follicle regeneration" refers to the renewal and/or activation of one or more regions and/or cell populations of the hair follicle so that the regenerated hair follicles can repeat the hair cycle. In particular, the regeneration occurs through the activity of epidermal stem cells.

Generally, the JAK inhibitors for use according to the invention will be in any of the pharmaceutical forms normally used in the cosmetics and dermatological fields, suitable for oral, transdermal, subcutaneous or topical administration to a site in need thereof, such as epidermal tissue, in particular the skin, the hair, or the scalp. Thus, it is also an object of the present invention to provide a pharmaceutical or cosmetic composition comprising a JAK inhibitor as described herein for use in any of the applications as described herein. The formulation of pharmaceutical and/or cosmetic compositions of JAK inhibitors as described herein and their subsequent administration (dosing) is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the condition or disease to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the condition or disease state is achieved.

A formulation suitable for oral administration may be in the form of pills, gelatine capsules, gels, emulsions, tablets, capsules or liquid solutions, in particular oral ampoules, for example. In particular, the active agent(s) according to the invention may be incorporated into any other forms of food supplements or enriched foods, for example food bars, or compacted or non-compacted powders. Preferably, the effective amount of JAK inhibitor present in the composition ranges from 10^{-12}% to 1% of the total weight of the composition, preferably from 10^{-9}% to 0.1%, and even more preferably from 10^{-7}% to 0.01% of the total weight of the composition.

Preferably, the invention features compositions comprising JAK inhibitors suitable for topical application to a site in need thereof, such as epidermal tissue, in particular the skin or the scalp, comprising, in a physiologically acceptable medium, at least an effective amount of a JAK inhibitor as described herein.

According to the invention, the term "physiologically acceptable medium" means a medium which is compatible with epidermal tissue, in particular the skin or the scalp, and/or its integuments (eyelashes, nails, hair) and/or the mucous membranes (lips).

The compositions according to the invention are cosmetic compositions or pharmaceutical compositions. The pharmaceutical compositions will preferably be dermatological compositions.

The effective amount of a JAK inhibitor as described herein present in the composition preferably ranges from 10^{-12}% to 1% of the total weight of the composition, 10^{-9}% to 0.1%, and even more preferably from 10^{-7}% to 0.01% of the total weight of the composition.

The composition may in particular be in the form of an aqueous, optionally gelled, solution, of a dispersion of the lotion type, optionally two-phase lotion, of an emulsion obtained by dispersion of a fatty phase in an aqueous phase (O/W) or conversely (W/O), or of a triple emulsion (W/O/W or O/W/O) or of a vesicular dispersion of the ionic and/or non-ionic type. These compositions are prepared according to the usual methods.

The JAK inhibitor for use according to the invention may also be formulated in compositions suitable for targeting to the deep layers of the epidermis, in particular for targeting to the basal layers of the epidermis or to the pilosebaceous unit. For example, the JAK inhibitor may be (i) encapsulated in a coating such as microspheres, nanospheres, oleosomes or nanocapsules, or (ii) compartmentalized in a fatty phase containing the main constituents of sebum (squalene, triglycerides, aliphatic waxes, cholesterol waxes and free cholesterol), or structural constituents present in proportions similar to those present in sebum. Particles called nanoparticles are in fact capable of crossing the superficial layers of the stratum corneum and/or of the follicular ostium, and of penetrating into the layers of the epidermis. The advantage of a composition in which the fatty phase mimics the composition of sebum is to allow better availability of the active agent at the target organ, i.e., at the sebaceous gland.

The composition may have, for example, the appearance of a white or coloured cream, of an ointment, of a milk, of a lotion, of a gel, of a serum, of a paste or of a foam, or may be in solid form (for example: stick) for application to the skin and/or the mucous membranes, such as the lips. It may also be in the form of a lotion of the aqueous, aqueous-alcoholic or oily solution type, of an oil-in-water or water-in-

oil or multiple emulsion, or of an aqueous or oily gel, or any other form suitable for application to the skin, the mucous membranes or the scalp.

In a known manner, the compositions according to the invention may also contain the adjuvants that are usual in the cosmetics or dermatological field, such as hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active agents, preservatives, antioxidants, solvents, fragrances, fillers, screening agents, pigments, odour absorbers and dyestuffs. The amounts of these various adjuvants are those conventionally used in the field under consideration, and are, for example, from 0.01 to 20% of the total weight of the composition. These adjuvants, depending on their nature, can be introduced into the fatty phase, into the aqueous phase or into the lipid vesicles. In any event, these adjuvants, and also the proportions thereof, will be chosen so as not to harm the desired properties of the JAK inhibitor.

When the composition according to the invention is an emulsion, the proportion of the fatty phase can range from 5 to 80% by weight, and preferably from 5 to 50% by weight relative to the total weight of the composition. The oils, the emulsifiers and the co-emulsifiers used in the composition in the form of an emulsion are chosen from those conventionally used in the field under consideration. The emulsifier and the co-emulsifier are present, in the composition, in a proportion ranging from 0.3 to 30% by weight, and preferably from 0.5 to 20% by weight relative to the total weight of the composition.

As oils that can be used in the invention, mention may be made of hydrocarbons of mineral or synthetic origin (liquid petroleum jelly, isohexadecane), oils of plant origin (apricot kernel oil, liquid fraction of karite butter, avocado oil, soybean oil), oils of animal origin (lanolin), synthetic oils (perhydrosqualene, pentaerythrityl tetraoctanoate), silicone oils (cylcopentasiloxane and cyclohexasiloxane) and fluoro oils (perfluoro-polyethers). Use may also be made, as fats, of fatty alcohols (cetyl alcohol or stearyl alcohol), of fatty acids (stearic acid) and of waxes (carnauba wax, ozokerite, beeswax).

As emulsifiers and co-emulsifiers that can be used in the invention, mention may be made, for example, of fatty acid esters of polyethylene glycol, such as PEG-100 stearate and PEG-20 stearate, and fatty acid esters of glycerol, such as glyceryl stearate.

As hydrophilic gelling agents, mention in particular may be made of carboxyvinyl polymers (carbomer), acrylic copolymers such as acrylate/alkyl acrylate copolymers, polyacrylamides, polysaccharides, natural gums and clays, and, as lipophilic gelling agents, mention may be made of modified clays such as bentones, metal salts of fatty acids, hydrophobic silica and polyethylenes.

As preservatives, mention may be made of esters of para-hydroxybenzoic acid, octane-1,2-diol, iodo-3-propynyl-2-butyl carbamate, phenoxyethanol and chlorhexidine gluconate.

As fillers, mention may be made, for example, of particles of polyamide (Nylon); microspheres of poly(methyl methacrylate); powders of ethylene-acrylate copolymer; expanded powders such as hollow microspheres, and in particular the microspheres made of a terpolymer of vinylidene chloride, of acrylonitrile and of methacrylate and sold under the name Expancel by the company Kemanord Plast; powders of natural organic materials such as starch powders, in particular powders of corn starch, of wheat starch or of rice starch, which may or may not be crosslinked, such as the powders of starch crosslinked with octenyl succinate anhydride; silicone resin microbeads such as those sold under the name Tospearl by the company Toshiba Silicone; silica; metal oxides such as titanium dioxide or zinc oxide; mica; and mixtures thereof.

As "solvents" mention may be made of hydrophilic organic solvents, lipophilic organic solvents, amphiphilic solvents or mixtures thereof. Among the hydrophilic organic solvents, mention may be made, for example, of linear or branched lower monoalcohols having from 1 to 8 carbon atoms, such as ethanol, propanol, butanol, isopropanol or isobutanol, optionally oxyethylenated polyethylene glycols, polyols such as propylene glycol, isoprene glycol, butylene glycol, glycerol, sorbitol and its derivatives, glycol ethers and propylene glycol ethers. As amphiphilic organic solvents, mention may be made of polyols such as propylene glycol derivatives. As lipophilic organic solvents, mention may be made, for example, of fatty esters.

In some embodiments, compositions comprising JAK inhibitors for use according to the invention can also be applied via transdermal delivery systems, which slowly release the active compound for percutaneous absorption. Permeation enhancers can be used to facilitate transdermal penetration of the active factors in the conditioned media. Transdermal patches are described in, for example and without limitation, US Patent No. 5,948,433; US Patent No. 5,407,713; US Patent No. 5,352,456; US Patent No. 5,336,168; US Patent No. 5,290,561.

Further, subcutaneous administration can refer to administration just beneath the skin (i.e. beneath the dermis). Generally, the subcutaneous tissue is a layer of fat and connective tissue that houses larger blood vessels and nerves. The size of this layer varies throughout the body and from person to person. The interface between the subcutaneous and muscle layers can be encompassed by subcutaneous administration. This mode of administration can be feasible where the subcutaneous layer is sufficiently thin so that the factors present in the compositions can migrate or diffuse from the locus of administration and contact the epidermal cells.

The compositions comprising a JAK inhibitor for use according to the invention may additionally comprise one or more other active agent. As "hydrophilic or lipophilic active agents" mention may be made of moisturizers, calmatives, depigmenting agents, anti-glycation agents, NO-synthase inhibitors, agents for stimulating the synthesis of dermal or epidermal macromolecules and/or for preventing their degradation, agents for stimulating fibroblast and/or keratinocyte proliferation or for stimulating keratinocyte differentiation, dermo-decontracting agents, tensioning agents, anti-pollution agents and/or free-radical scavengers, photoprotective agents, repairing and/or cicatrizing agents, antidandruff agents, and mixtures thereof.

According to further embodiments, a method is provided for activating epidermal stem cell populations and/or epidermal progenitor cells, the method comprising contacting epidermal tissue or cells with an effective amount of a JAK inhibitor, in particular a JAK inhibitor that inhibits JAK3.

Also, a method is provided for stimulating hair growth and/or treating a hair-loss disorder in a subject in need thereof, the method comprising administering to a subject in need thereof an effective amount of a JAK inhibitor, in particular a JAK inhibitor that inhibits JAK3, formulated in a physiological acceptable medium.

A variety of subjects are treatable according to the methods of the invention. Generally such subjects are mammals or mammalian, where these terms are used broadly to describe organisms which are within the class Mammalia, including the orders carnivore (e.g., dogs and cats), rodentia (e.g., mice, guinea pigs, and rats), and primates (e.g., humans, chimpanzees, and monkeys). In many embodiments, the subjects will be humans.

Also, a method for skin regeneration or for wound healing is provided, the method comprising applying to a site in need thereof an effective amount of a JAK inhibitor, in particular a JAK inhibitor that inhibits JAK3, formulated in a physiological acceptable medium.

As used herein, the expression “a site in need thereof” refers to the site of injury or the site for intended repair or regeneration of skin.

The following examples are intended to promote a further understanding of the present invention. While the present invention is described herein with reference to illustrated embodiments, it should be understood that the invention is not limited hereto. Those having ordinary skill in the art and access to the teachings herein will recognize additional modifications and embodiments within the scope thereof. Therefore, the present invention is limited only by the claims attached herein.

EXAMPLES

Example 1. The hair follicle stem cell population increases during aging

Keratin-15 (Krt-15) positive hair follicle cells are one of the best-characterized stem cell populations in the skin. Specifically, studies using Krt-15-promoter reporter mouse models have demonstrated that these cells possess stem cell properties, as they are label retaining cells with multipotent capacity, contributing to hair follicle cycling, sebaceous gland function and wound repair (Morris et al. 2004; Ito et al. 2005; Petersson et al. 2011). In agreement with published work, whole-mount immunostaining for GFP in the tails of young Krt-15-GFP reporter mice confirmed these cells as a subset of the Krt-15 protein-expressing fraction, with the GFP reporter identifying the most primitive compartment (Morris et al. 2004) (Fig. 1a). Although expression is seen in the sebaceous gland and the epidermis, immunohistochemical and FACS analysis supported previous studies demonstrating significant overlap with the $CD34^+$ population of stem cells in the bulge region (data not shown). However, temporal analyses of Krt-15-GFP expression in the bulge region of the hair follicle revealed a significant increase in GFP with respect to Krt-15 protein in aged mice (Fig. 1a and b), as well as an expansion of the absolute number of GFP^+ cells per hair follicle unit (Fig. 1c). Corroborating these observations, we also documented temporal enrichment of GFP^+ cells in flowcytometric analyses of aged back skin epidermis (Fig. 1d). As has been previously reported (Giangreco et al. 2008), we did not observe any significant differences in the overall $CD34^+/CD49f^+$ or Krt-15 protein-positive populations (Fig. 1e and f, respectively). We did, however, observe that the increasing Krt-15-GFP^+ cells co-expressed CD34 (Fig. 1g), thus highlighting a previously undocumented increase in this hair follicle stem cell population during aging.

Example 2. The functional capacity of the hair follicle stem cell population declines with aging

To test if these accumulating aged Krt-15-GFP stem cells retained functional capacity, we FACS-purified GFP-positive and negative epidermal fractions and plated them in equal numbers to assess clonogenic capacity (Barrandon and Green 1987). In agreement with earlier reports (Morris et al. 2004), young (3 month) GFP^+ cells gave rise to larger and significantly greater numbers of colonies compared to GFP^- control cells (Fig. 2a, left panels). Strikingly, we observed a significantly diminished colony forming ability of aged (18 month) GFP^+ cells cultured under identical conditions (Fig. 2a-c). Similarly, parallel studies using FACS-isolated triple-positive ($CD34^+/CD49f^+/GFP^+$) stem cells (Fig. 2d), as well as the total $CD34^+/CD49f^+$ population (data not shown) also revealed an age-associated decline in functional capacity, thus reinforcing the notion that bona-fide stem cells are indeed impaired with advanced age. We subsequently tested if these aged stem cells were functionally impaired *in vivo*. First, we subjected

young and old Krt-15-GFP mice to ionizing radiation and measured the change in stem cell number in response to exogenous low-level DNA damage (Davies et al. 2008; Liang et al. 2011). Surprisingly, whereas the Krt-15-GFP stem cells in young mice exhibited an approximately two-fold increase in response to acute DNA damage there was no change in old mice (Fig. 2e). Similar results were also seen for the Krt-15-GFP$^+$/CD34$^+$/CD49f$^+$ population (data not shown) suggesting that aged stem cells are either unable to respond to the stress or become depleted as a result. To examine this observation in greater detail, we treated shaved, dorsal back skin with 12-O-tetradecanoylphorbol-13-acetate (TPA), an inducer of stem cell activation and epidermal hyperproliferation. Interestingly, at the tissue level, aged skin was not able to tolerate TPA as well as young skin, and rapidly developed skin lesions (data not shown). In agreement with our earlier data, counting of individual GFP$^+$ stem cells in the hair follicle bulge in untreated young and old dorsal back skin revealed an age-associated increase in absolute cell number with age (Fig. 2f). However, upon treatment with TPA, whereas young skin exhibited a significant increase in stem cell number in response to stimulus, aged skin displayed the opposite trend, with depletion of both Krt-15-GFP and CD34 immunoreactivity (Fig. 2f-g). Altogether, this demonstrates an inherent inability of aged stem cells to be maintained following substantial cellular stress.

Example 3. Comparative expression analysis of hair follicle stem cell population during aging.

To gain deeper insight into the molecular mechanisms underlying these age-related changes, we performed high-throughput RNA-sequencing on 3- and 18-month Krt-15-GFP cells freshly isolated from the skin. Importantly, expression (FPKM) values generated by sequencing, and further selectively validated by qRT-PCR, demonstrated that with age, the GFP$^+$ stem cell population retains, and possibly increases the relative expression of a core stem cell signature (Tumbar et al. 2004; Lien et al. 2011) (Figs. 3, 4). Interestingly, while the core signature of these cells increased, we observed little change or even possible decreases in the alternate fate-signatures (Tumbar et al. 2004; Lien et al. 2011), namely interfollicular epidermis and sebaceous gland, suggesting there may be fate changes within this population with age (Fig. 3).

Unbiased, global analyses of transcript expression in highly purified Krt-15-GFP cells revealed substantial changes in many genes and biological processes (Figure 5a, Fig. 6). Given that stem cell functional decline has been linked with changes in key signalling pathways (Silva-Vargas et al. 2005; Brack et al. 2007), we initially focused on these for subsequent analyses. On the basis of Gene Ontology (GO) annotations, we manually extracted transcripts associated with positive and negative regulation of Jak-Stat, Wnt, Hedgehog, Tgf-β and Notch signaling to survey canonical signal transduction activity. Interestingly, two pathways (Jak-Stat and Notch) stood out as being significantly altered with age, as

evidenced by a robust inverse relationship between positive and negative signalling regulators (Fig. 5b). We also validated many of these gene-expression changes by qRT-PCR in independent biological replicates (Fig. 5c). Collectively, our deep-sequencing results demonstrate that while Krt-15-GFP cells maintain a stem-like signature during aging, they also exhibit marked alterations in critical signal transduction cascades. In particular, the transcriptional change in regulators of Jak-Stat signalling represented the signalling pathway that was most prominently deregulated in our analysis.

Example 4. JAK inhibitors rescue aged stem cell activity *in vitro*

Taking into account the above observations, we hypothesized that repression of Jak-Stat signalling may have potentially growth-promoting effects in aged epidermis. To address this, we generated clonogenic cultures from aged (18 month) wild type mice and treated these with the protein kinase inhibitor pyridone-6 (Jak Inhibitor I, Jaki), a global inhibitor of the Jak-Stat pathway. Strikingly, this resulted in a robust rescue of aged stem cell clonogenic activity, evidenced by more abundant and significantly faster growing colonies compared to vehicle-treated control cultures that maintained expression of prototypical epidermal markers including p63, Keratin 14 and CD49f (Fig. 7a, Fig. 8a). This rescue effect was not seen when we tested inhibitors of other pathways that showed little change in our transcriptome analysis (Wnt, Hedgehog and Notch), or the Wnt agonist BIO (Fig. 7a and Fig. 8b), highlighting the functional significance of Jak-Stat transcript changes derived from our sequencing data. Rescue of aged colony forming ability was also seen with two other inhibitors of the Jak-Stat pathway (the Jak3 inhibitor Tofacitinib and the Jak1/2 inhibitor Ruxolitinib), highlighting the specificity of these results (Fig. 9). This effect of Jak kinase inhibition was also evident when we treated aged GFP^+ or $GFP^+/CD34^+/CD49f^+$ cultures, indicating that Jak-inhibition has a pro-proliferative effect directly on aged stem cells (Fig. 7b) including on cells derived from mice exposed to γ-irradiation *in vivo* (Fig. 10b). Interestingly, non-bulge stem cell populations ($GFP^+/ CD34^-$) also exhibited an age-associated decrease in proliferation that was rescued with the inhibitor, suggesting that Jak kinase suppression may have effects on non-stem cells or other stem cell populations within the culture (Fig. 10a).

Example 5. JAK inhibitors restore stem cell functioning and hair follicle status *in vivo*

Having identified inhibition of Jak-Stat signalling as a potent means of restoring aspects of stem cell function *in vitro*, we tested its potential biological relevance *in vivo*. Consistent with our clonogenicity results, a one-week topical Jak inhibitor (Jaki) regimen on 18-month tail epidermis resulted in a substantial increase in the number of active hair follicles (HFs). By whole-mount immunohistochemistry, we observed an increase in Ki67 and CCAAT displacement protein (CDP) expression, both of which are associated with actively cycling hair follicles (Fig. 7c). Quantitatively, we

observed fewer than 5% anagen (active) HFs in vehicle-treated epidermis. However, this increased to over 35% when exposed to the inhibitor (Fig. 7d). This effect seemed to be independent of the stage of the mature hair follicle when treated, as follicles were largely in telogen (resting) at both time points examined using anagen hair follicle counting and analysis of relevant signalling pathways (data not shown). Furthermore, histological examination of shaved back skin treated with vehicle or Jak inhibitor, also revealed significant hair follicle re-activation (Fig. 7e). However, upon further investigation, we observed a marked depletion in both Krt-15-GFP and CD34 immunoreactivity in Jak-inhibitor-treated samples, suggesting that, while aged hair follicles are capable of being stimulated into an active state, enhanced proliferative/activating signals may ultimately drive the stem cells to exhaustion and depletion (Fig. 7e). Taken together, these data uncover a complex relationship between epidermal stem cell proliferation and self-renewal in aged skin and show that aspects of this relationship can be partially restored through inhibition of the Jak-Stat pathway.

Materials and Methods to the Examples

Animal Use

Aged keratin-15-GFP (Morris et al. 2004) or wildtype C57Bl6/J mice were housed in accordance with the CEEA (Ethical Committee for Animal Experimentation) of the Government of Catalonia. For chemical epidermal activation assays, shaved back or tail skin was treated either once or three times over one week with 20 nM 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma-Aldrich). Skin samples were harvested the day following the final treatment and processed as described below. Ionizing radiation (IR) experiments were performed by subjecting mice to 5Gy whole-body irradiation followed by flow cytometric analysis of single-cell epidermal preparations at the indicated time points. For *in vivo* pyridone-6 (Jak inhibitor I, Calbiochem) assays, a 1mM inhibitor preparation (in DMSO/acetone) was applied to tail skin and harvested as described for TPA experiments.

Primary mouse keratinocyte clonogenicity assays

Primary mouse keratinocytes were harvested from dorsal back skin of aged mice as described previously (Jensen et al. 2010). Chemical and cytokine treatments were initiated approximately 8h following establishment of the co-culture in order to allow for equal initial seeding numbers. All cytokines were purchased from Peprotech and used as follows: Il-1α (0.4-4ng/ml), Il-1ra (5-50ng/ml), GM-CSF (1-10ng/ml), ICAM-1 (4-40ng/ml), BLC (1-10ng/ml). Chemical activators/inhibitors were used as follows: pyridone-6 (Calbiochem, 50-500nM), DAPT (Sigma, 1-10uM), BIO (Cayman Chemical, 500nM-1uM), IWR-1 endo and exo (Cayman Chemical, 1-5uM), Cyclopamine (Calbiochem, 1-5uM), Tofacitinib (Selleck Chemicals, 80-400nM), Ruxolitinib (Selleck Chemicals, 80-400nM). To visualize

clonogenic keratinocyte expansion, co-cultures were fixed in 10% formalin, stained with a crystal violet/methanol, and imaged using conventional scanning and imaging tools.

Whole-mount immunofluorescence

Preparation of tail skin and whole-mount staining was performed as previously described (Braun et al. 2003). Primary antibodies were incubated overnight at room temperature and used at the following concentrations: anti-GFP (Invitrogen, 1:200); anti-Ki67 (Abcam, 1:200); anti-keratin-15 (NeoMarkers, 1:200); and anti-CDP (Santa Cruz, 1:100). Alexa-conjugated secondary antibodies (Molecular Probes, 1:500) were used for two hours at room temperature. Nuclei were then stained with DAPI (Roche, 1:10000), and epidermal sheets were mounted in Mowiol. Pictures were acquired with a Leica TCS SP5 confocal microscope and when applicable, quantified using ImageJ software.

Immunohistochemistry

Back skin and/or tail skin was fixed in 10% NBF (Sigma-Aldrich) overnight, washed in PBS, then embedded in paraffin. Deparaffinized sections were boiled for 10 min in 0.01 M citric acid for antigen retrieval. Primary antibodies were incubated overnight at 4 °C, and secondary antibodies were incubated for 2h at room temperature in 0.25% gelatin/PBS. Nuclei were stained with DAPI (1:10000, Roche), and the slides were mounted in Vectashield (Vector labs). Primary antibodies were used at the following dilutions: anti-keratin 15 (MS-1068-P1, NeoMarkers, 1:200); anti-Ki67 (ab15580, Abcam, 1:200); anti-GFP (A11122, Invitrogen 1:200); anti-CD34 (560233, BD Biosciences, 1:50); anti-ICAM-1 (4915, Cell Signaling, 1:50); anti-Stat3 (9132, Cell Signaling, 1:50); and anti-p-Stat3 (Tyr705) (9145, Cell Signaling, 1:50). Alexa-conjugated secondary antibodies (Molecular Probes) were all used at a dilution of 1:500. Pictures were acquired with a Leica DMI 6000B or a Leica TCS SP5 confocal microscope.

Flow cytometry

Epidermal cells from back skin of Keratin-15-GFP or wild type C57Bl6/J mice were isolated as described above. Cell suspensions were incubated for 30min on ice with the following antibodies at the given dilutions: APC-conjugated anti-CD34 (1:200, clone RAM34, BD Pharmingen), and FITC-conjugated anti-α6-integrin (1:200, CD49f clone NKI-GoH3, Serotec). Keratinocytes were sorted on the basis of single cellularity, viability (DAPI), GFP positivity (Keratin-15+) and/or CD34/CD49f status. FACS purification of was performed on a FACS Aria system equipped with FACS DiVa software (BD Bioscience). Sorted cells were collected in keratinocyte medium supplemented to 50% FBS, and either plated for clonogenic assays or pelleted and stored at -80C for RNA isolation. FACS analysis was performed using LSRII FACS Analysers (BD Biosciences) and analysed using Flowjo software.

RNA-seq and analysis

Total RNA was isolated from pooled, FACS-sorted GFP+ cells using the RNeasy Micro Kit (Qiagen). RNA-seq libraries were prepared using an Illumina RNA prep kit and sequenced using the Illumina HiSeq2000 platform. A total of 233 million 46-bp paired-end reads were mapped to the Mus musculus genome (NCBIM37.57) by using Tophat aligner (version 1.3.0) (Trapnell et al. 2009), a RNAseq mapper specifically designed for detecting splice junctions between exons and based on the fast NGS mapper Bowtie (Langmead et al. 2009). Tophat parameters were set to default with mate pair distance of 150. Final transcript levels of all mouse Ensembl known genes (v57) were calculated in unit of fragments per kilobase of exon per million of fragments mapped (FPKM) by counting the number of mapped and spliced reads to exons, normalized by length of the exons and averaged over all used exons for each transcript. On average, ~81% of the total reads (equivalent to 188 million sequences) were mapped to the genome or to the splice junctions. Of these, 85,6% mapped uniquely, 8,88% 2 times, 2,8% 3 times, and 2,9% from 4 to 20 times. Cuffdiff tool from the Cufflinks package (version 1.0.3) (Roberts et al. 2011) was then used to detect the transcripts whose expression statistically changes between 3- and 18-month Krt-15-GFP+ cells.

RT–qPCR

Total RNA from cultured or FACS-sorted cells was purified using the RNeasy Micro or Mini Kit (Qiagen). Equal amounts of RNA were reverse-transcribed using random hexamer priming and Superscript III (Invitrogen). RT–qPCR was performed with SYBR Green Master Mix (Roche) and gene-specific primers (sequences provided in Table 1) using a Light Cycler 480 Instrument (Roche). Relative levels of expression were determined using the $\Delta\Delta C_t$ method relative to the housekeeping gene actin.

Table 1

PRIMER NAME	PRIMER SEQUENCE	LENGTH (BP)	SEQ ID NO
Q-BETA-ACTIN-FWD	GATCTGGCACCACACCTTCT	20	1
Q-BETA-ACTIN-REV	GGGGTGTTGAAGGTCTCAAA	20	2
Q-MK14-FWD	CGCCGCCCCTGGTGTGG	17	3
Q-MK14-REV	ATCTGGCGGTTGGTGGAGGTCA	22	4
Q-MK5-FWD	AACATTTTGGGGTCTGGGTCAC	22	5
Q-MK5-REV	GGCCCACAGAGACTGCTTCTTT	22	6
Q-MLRIG1-FWD	ACTCAAGAGTCTGCGGGTCT	20	7
Q-MLRIG1-REV	TCCTCGATTGTACCCGAGAT	20	8
Q-MK15-FWD	GGAAGAGATCCGGGACAAA	19	9
Q-MK15-REV	TGTCAATCTCCAGGACAACG	20	10
Q-MCD34-FWD	GGGTAGCTCTCTGCCTGATG	20	11
Q-MCD34-REV	TCCGTGGTAGCAGAAGTCAA	20	12
Q-MCCL5-FWD	ATATGGCTCGGACACCACTC	20	13
Q-MCCL5-REV	GCACTTGCTGCTGGTGTAGA	20	14
Q-MHES1-FWD	AAACGAAAATGCCAGCTGAT	20	15
Q-MHES1-REV	GTCTTTGGTTTGTCCGGTGT	20	16
Q-MIL24-FWD	CCTGACCTGGATGCAGAAAT	20	17
Q-MIL24-REV	CTTGAGGACAGCAGGGATGT	20	18
Q-MIGF1-FWD	CACAAACTCACCACCCTGTG	20	19
Q-MIGF1-REV	TGACATATTGCCCCCATTTT	20	20
Q-MLEP-FWD	TGAAAGGGTGAGGCATTTTC	20	21
Q-MLEP-REV	TCCAAATGTTCCGGAAAGAG	20	22
Q-MJAK2-FWD	GTCCACCCGTGGAATTTATG	20	23
Q-MJAK2-REV	GCAATCTTCCGTTGCTCTTC	20	24
Q-MISL1-FWD	ACGTGCTTTGTTAGGGATGG	20	25
Q-MISL1-REV	TGAAGCCTATGCTGCACTTG	20	26
Q-MCAV1-FWD	GCTAAACCGAGACTGCCAAG	20	27
Q-MCAV1-REV	AAGGTCGAGCTTCACAGCAT	20	28
Q-MSOCS1-FWD	ACTTCTGGCTGGAGACCTCA	20	29
Q-MSOCS1-REV	CCCAGACACAAGCTGCTACA	20	30
Q-MSOCS2-FWD	CCCAACCTAGTGCCATTGTT	20	31
Q-MSOCS2-REV	TCCGTGGTCAGACAATTCAA	20	32
Q-MSOCS3-FWD	ATTCACCCAGGTGGCTACAG	20	33
Q-MSOCS3-REV	GCCAATGTCTTCCCAGTGTT	20	34

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CLAIMS

1. A JAK inhibitor for use in activation of a population of epidermal stem cells and/or progenitor cells.
2. The JAK inhibitor according to claim 1, wherein the population of epidermal stem cells and/or progenitor cells is a population of hair follicle stem cell and/or progenitor cells.
3. The JAK inhibitor according to any of claims 1 to 2, wherein the JAK inhibitor is an inhibitory agent or a small molecule.
4. The JAK inhibitor according to any of claims 1 to 3, wherein the JAK inhibitor inhibits JAK3.
5. The JAK inhibitor according to any of claims 1 to 4 for use in hair follicle regeneration.
6. The JAK inhibitor according to claim 5 for stimulating hair growth and/or for use in treatment of a hair-loss disorder.
7. The JAK inhibitor according to any of claims 1 to 4 for use in skin regeneration.
8. The JAK inhibitor according to any of claims 1 to 4 for use in wound healing.
9. The JAK inhibitor according to any of claims 1 to 8 wherein the JAK inhibitor is formulated in a physiologically acceptable medium.
10. The JAK inhibitor according to claim 9 wherein said physiologically acceptable medium is suitable for topical application.
11. The JAK inhibitor according to any of claims 1 to 10 wherein the population of epidermal stem cells is a human stem cell population.
12. A method for activating a population of epidermal stem cells and/or progenitor cells, the method comprising contacting epidermal tissue or cells with an effective amount of a JAK inhibitor, in particular a JAK inhibitor that inhibits JAK3.
13. A method for stimulating hair growth and/or treating a hair-loss disorder in a subject in need thereof, the method comprising applying to a subject in need thereof an effective amount of a JAK inhibitor, in particular a JAK3 inhibitor, formulated in a physiological acceptable medium.
14. A method for skin regeneration or for wound healing, the method comprising applying to a site in need thereof an effective amount of a JAK inhibitor, in particular a JAK3 inhibitor, formulated in a physiological acceptable medium.
15. The method according to any of claims 13 or 14 wherein said physiologically acceptable medium is suitable for topical application.

Figure 1

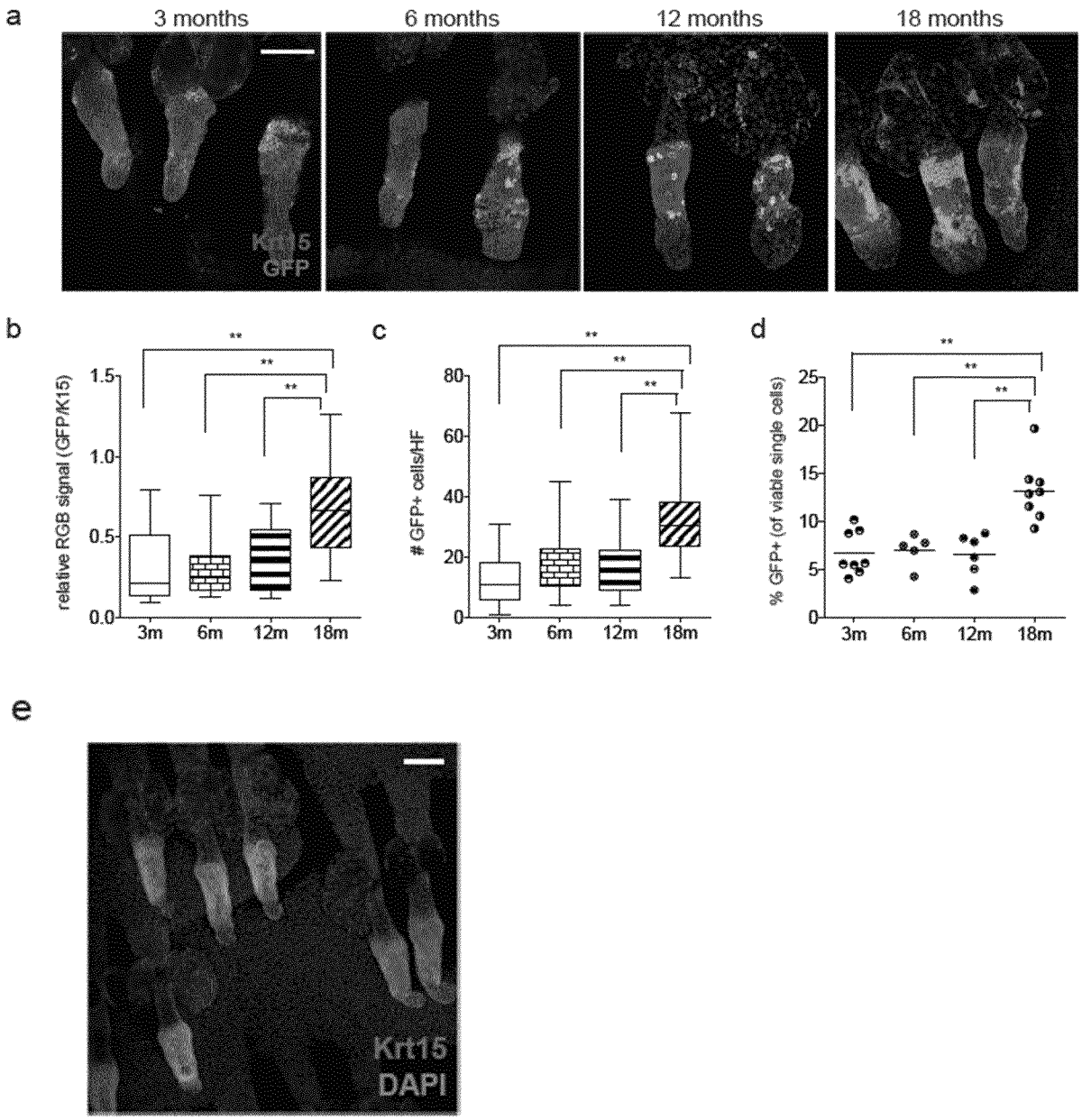

a
3 months
6 months
12 months
18 months
Krt15
GFP
b
relative RGB signal (GFP/K15)
1.5
1.0
0.5
0.0
3m
6m
12m
18m
**
c
GFP+ cells/HF
80
60
40
20
0
d
% GFP+ (of viable single cells)
25
20
15
10
5
0
e
Krt15
DAPI

Figure 1 continued

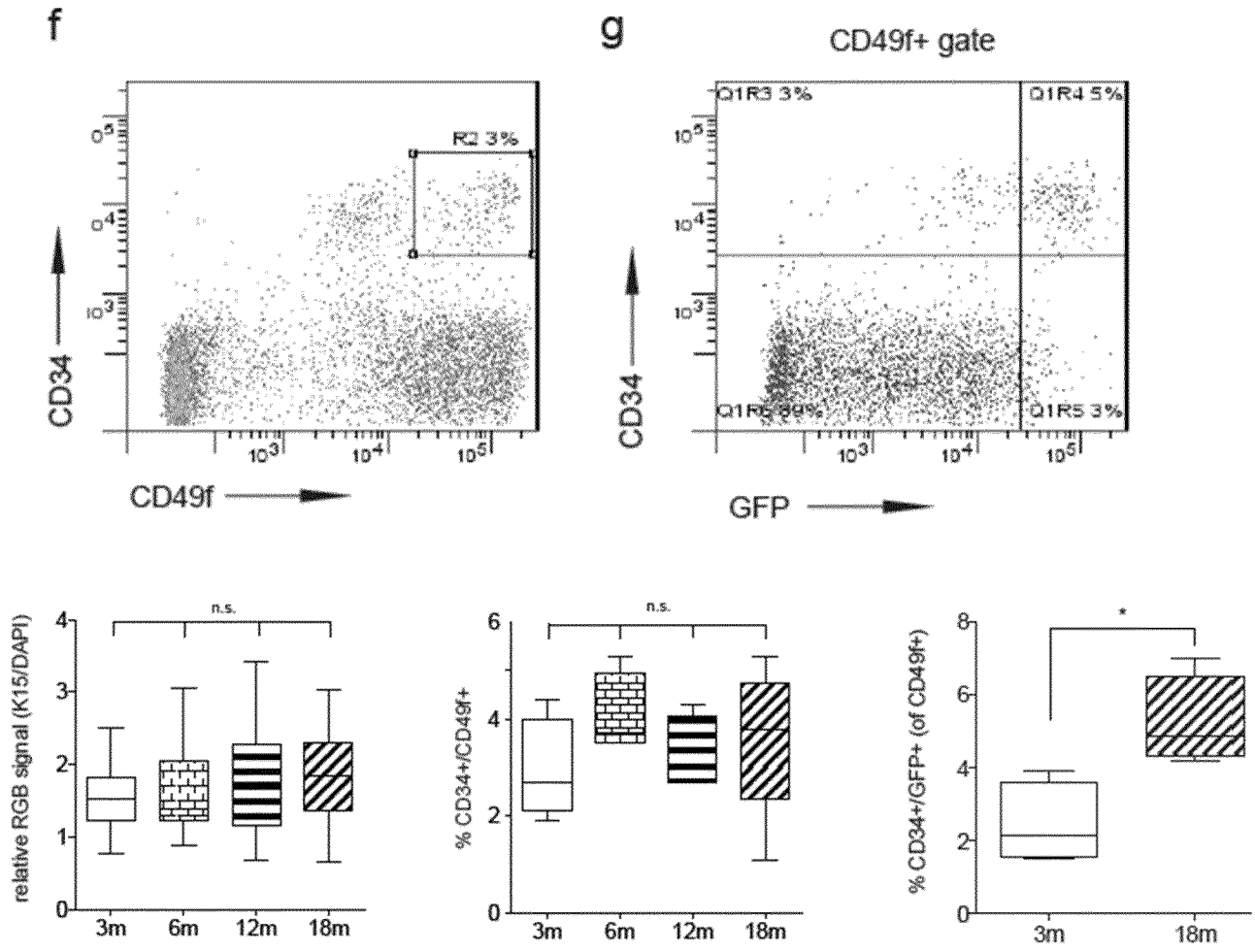


Figure 2

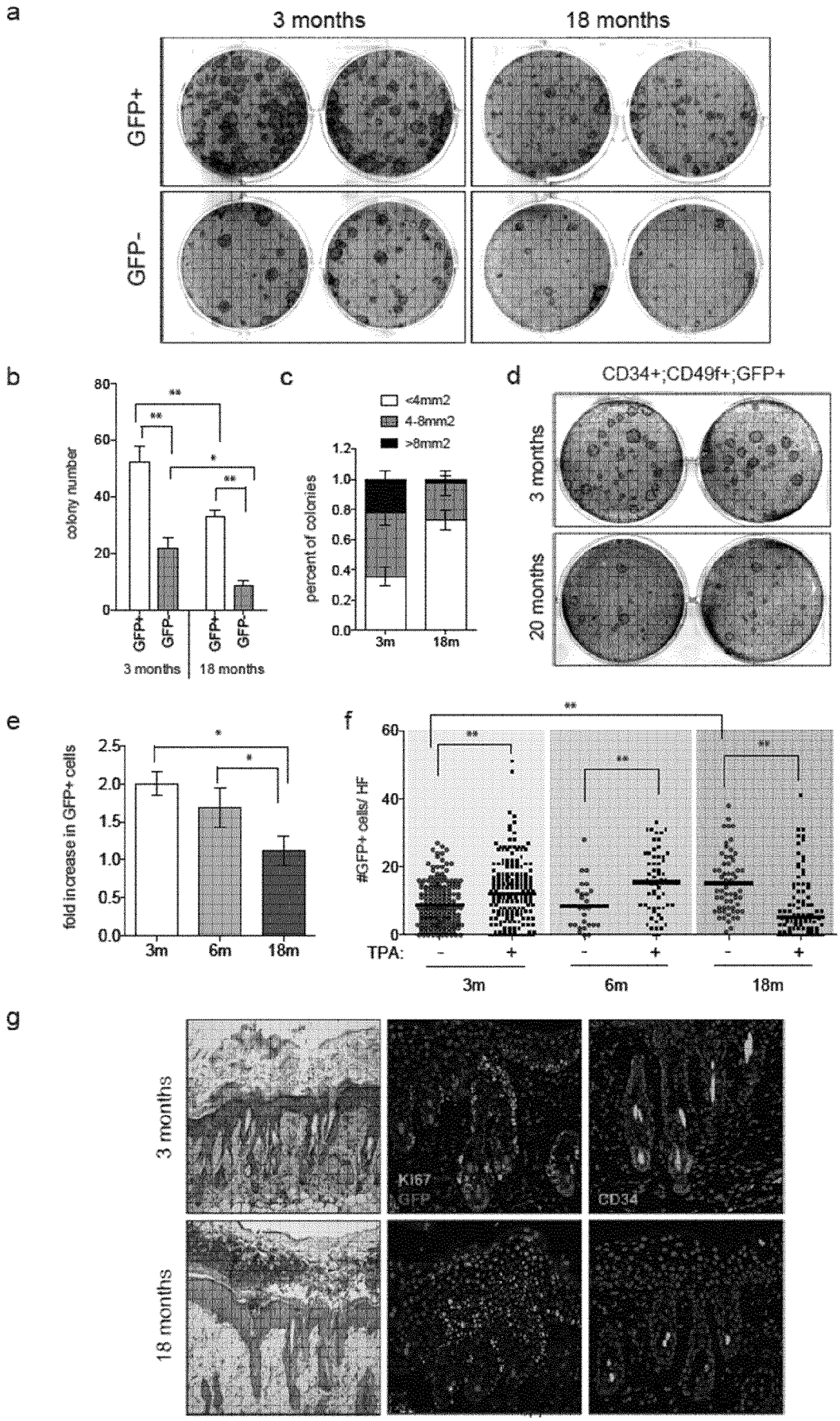

a
3 months
18 months
GFP+
GFP-
b
colony number
GFP+
GFP-
3 months
18 months
c
<4mm2
4-8mm2
>8mm2
percent of colonies
3m
18m
d
CD34+;CD49f+;GFP+
3 months
20 months
e
fold increase in GFP+ cells
3m
6m
18m
f
#GFP+ cells/ HF
TPA:
3m
6m
18m
g
3 months
18 months
KI67
GFP
CD34

Figure 3

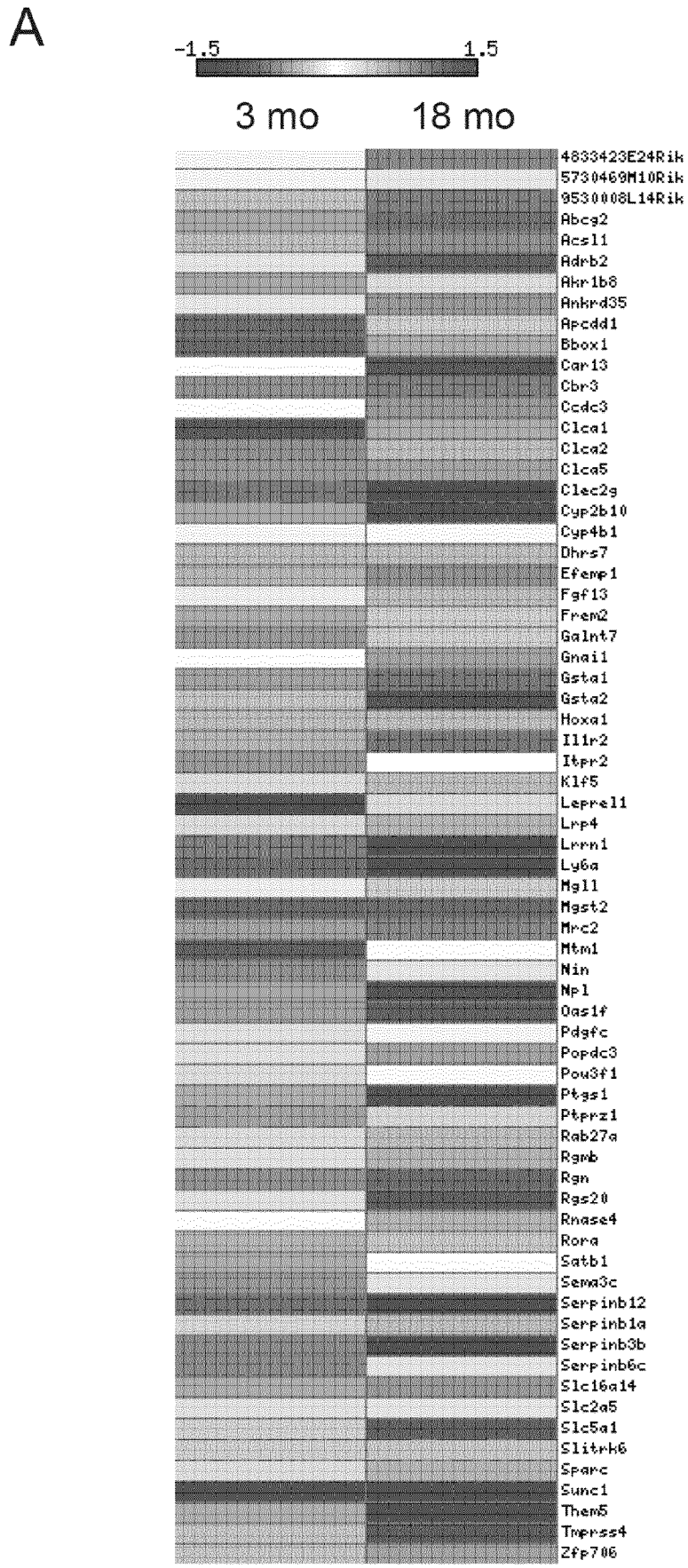
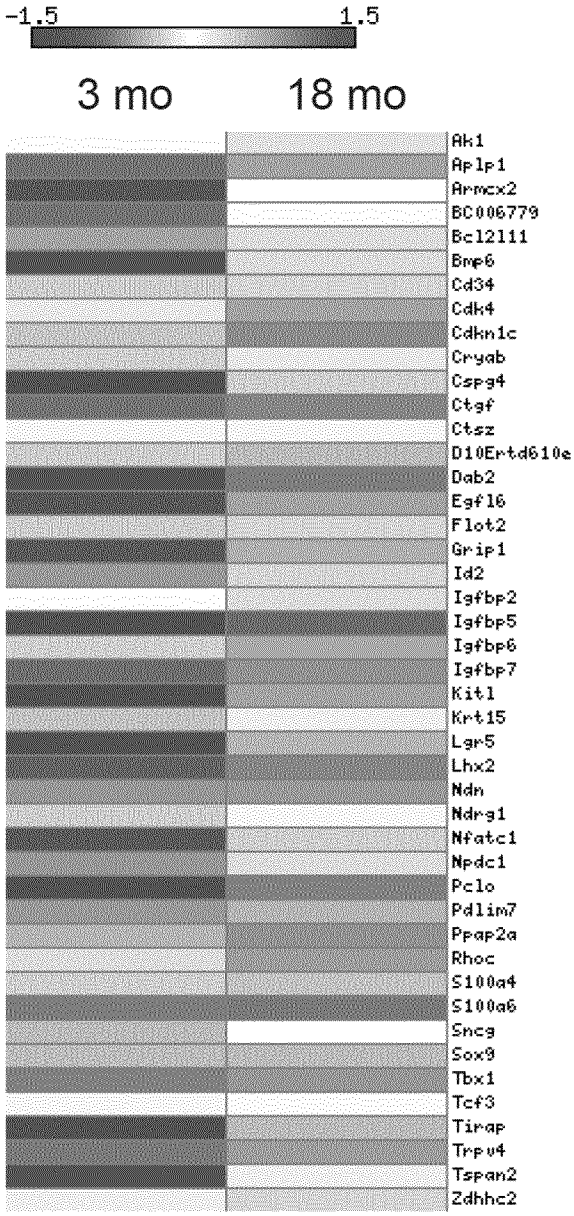
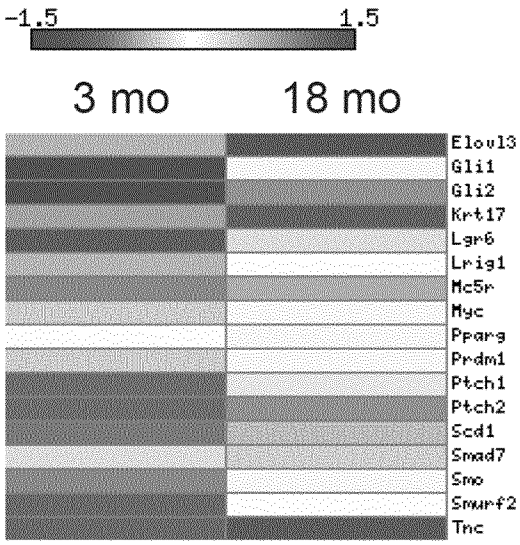

A
-1.5
1.5
3 mo
18 mo
4833423E24Rik
5730469M10Rik
9530008L14Rik
Abcg2
Acsl1
Adrb2
Akr1b8
Ankrd35
Apcdd1
Bbox1
Car13
Cbr3
Ccdc3
Clca1
Clca2
Clca5
Clec2g
Cyp2b10
Cyp4b1
Dhrs7
Efemp1
Fgf13
Frem2
Galnt7
Gnai1
Gsta1
Gsta2
Hoxa1
Il1r2
Itpr2
Klf5
Leprel1
Lrp4
Lrrn1
Ly6a
Mgll
Mgst2
Mrc2
Mtm1
Nin
Npl
Oas1f
Pdgfc
Popdc3
Pou3f1
Ptgs1
Ptprz1
Rab27a
Rgmb
Rgn
Rgs20
Rnase4
Rora
Satb1
Sema3c
Serpinb12
Serpinb1a
Serpinb3b
Serpinb6c
Slc16a14
Slc2a5
Slc5a1
Slitrk6
Sparc
Sunc1
Themis
Tmprss4
Zfp706

Figure 3 continued

B



C



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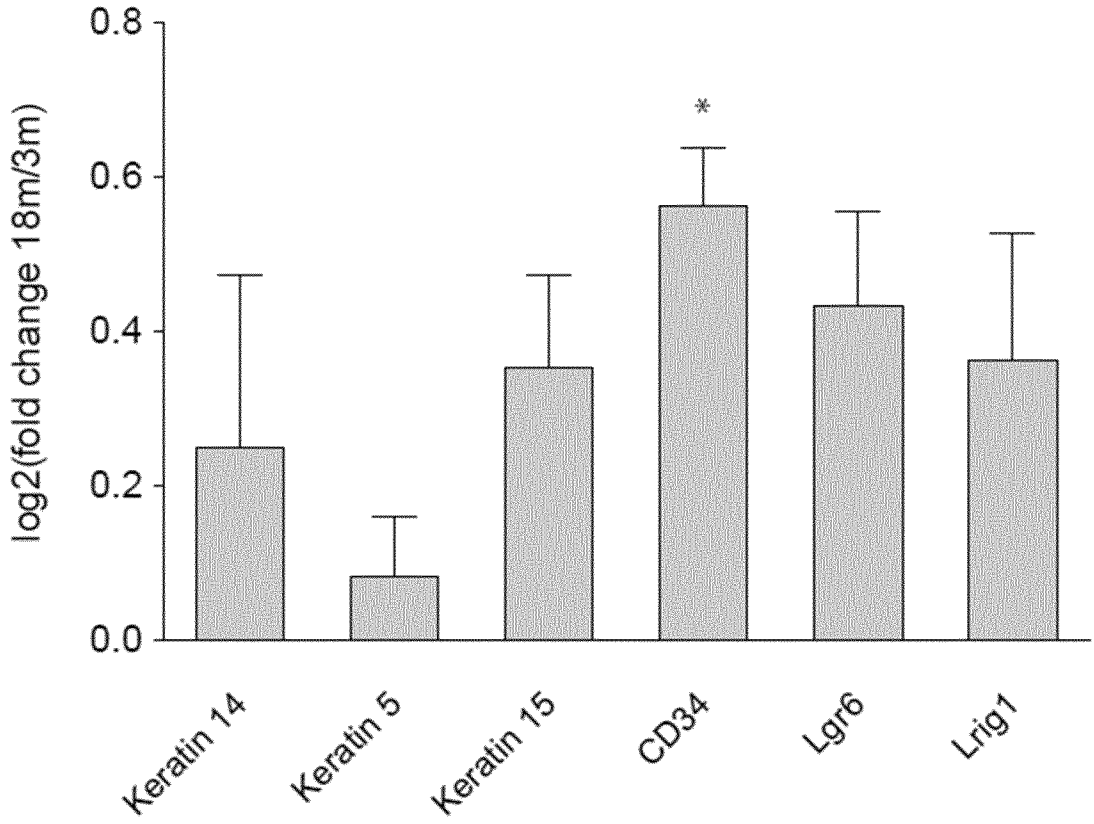

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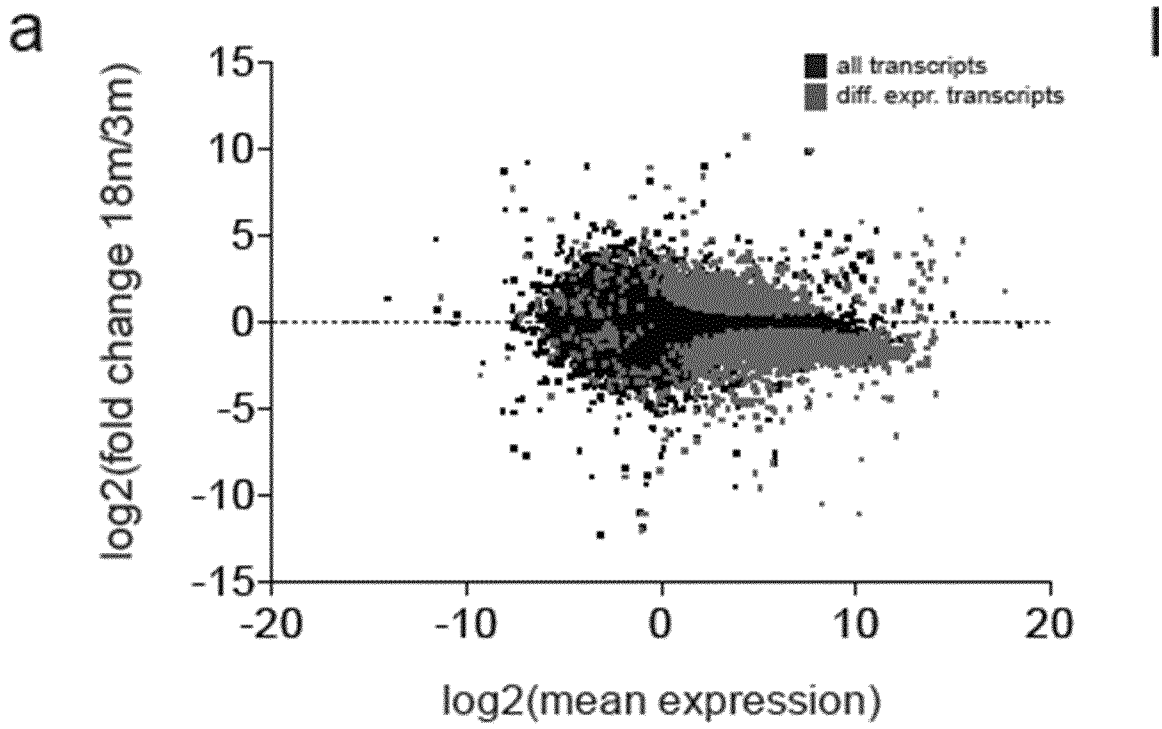
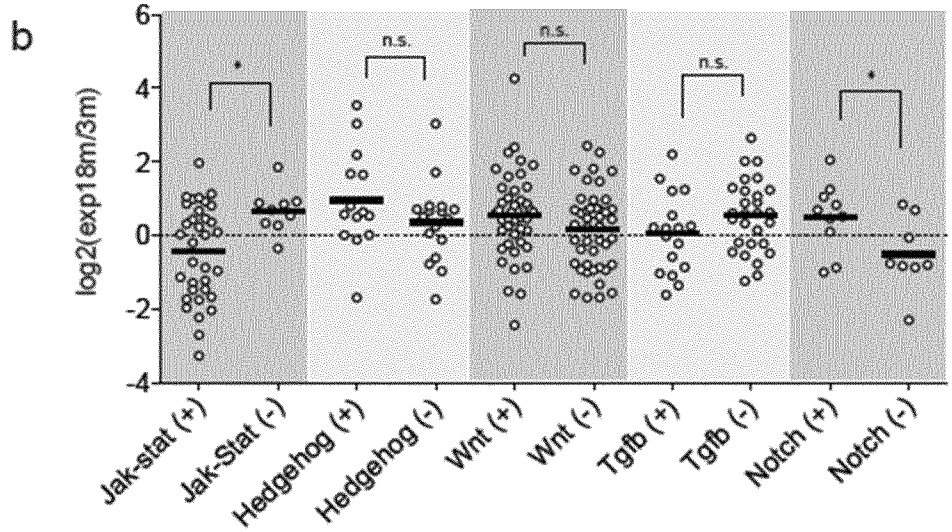


Figure 5 continued



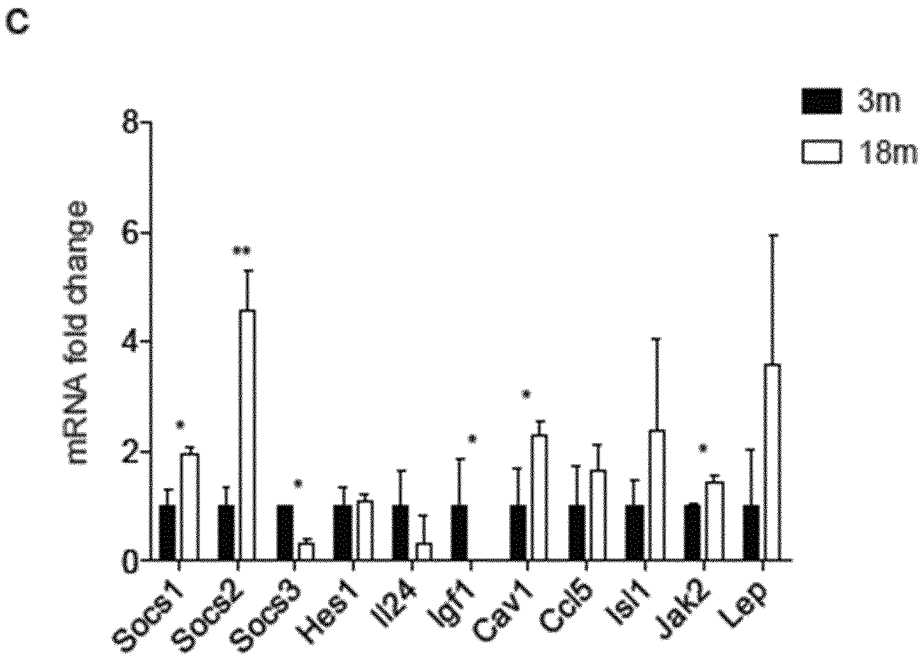


Figure 6

A

Top 20 upregulated Gene Ontology processes

GO process	Description	P-value
GO:0044260	cellular macromolecule metabolic process	8.33E-08
GO:0043170	macromolecule metabolic process	5.66E-06
GO:0018193	peptidyl-amino acid modification	7.30E-06
GO:0051171	regulation of nitrogen compound metabolic process	1.08E-05
GO:0043412	macromolecule modification	1.22E-05
GO:0006464	protein modification process	1.38E-05
GO:0019219	regulation of nucleobase-containing compound metabolic process	1.97E-05
GO:2001141	regulation of RNA biosynthetic process	2.38E-05
GO:0034645	cellular macromolecule biosynthetic process	2.73E-05
GO:0016568	chromatin modification	2.88E-05
GO:0006355	regulation of transcription, DNA-dependent	2.96E-05
GO:0044267	cellular protein metabolic process	3.15E-05
GO:0009059	macromolecule biosynthetic process	3.66E-05
GO:0006996	organelle organization	4.09E-05
GO:0032774	RNA biosynthetic process	4.33E-05
GO:0006351	transcription, DNA-dependent	5.43E-05
GO:0051252	regulation of RNA metabolic process	5.69E-05
GO:0051253	negative regulation of RNA metabolic process	6.38E-05
GO:0006325	chromatin organization	6.77E-05
GO:0010629	negative regulation of gene expression	9.13E-05

B

Top 20 downregulated Gene Ontology processes

GO process	Description	P-value
GO:0051707	response to other organism	5.37E-07
GO:0009607	response to biotic stimulus	3.78E-06
GO:0051704	multi-organism process	3.78E-06
GO:0048525	negative regulation of viral reproduction	9.10E-06
GO:0048821	erythrocyte development	2.56E-05
GO:0006952	defense response	1.18E-04
GO:0045071	negative regulation of viral genome replication	1.43E-04
GO:0042742	defense response to bacterium	1.56E-04
GO:0010951	negative regulation of endopeptidase activity	1.57E-04
GO:0009615	response to virus	2.07E-04
GO:0032355	response to estradiol stimulus	2.12E-04
GO:0010466	negative regulation of peptidase activity	2.28E-04
GO:0002682	regulation of immune system process	2.95E-04
GO:0006955	immune response	3.30E-04
GO:0002252	immune effector process	4.68E-04
GO:0030216	keratinocyte differentiation	4.76E-04
GO:0002376	immune system process	4.95E-04
GO:0002695	negative regulation of leukocyte activation	5.26E-04
GO:0051250	negative regulation of lymphocyte activation	5.26E-04
GO:0051346	negative regulation of hydrolase activity	6.07E-04

Figure 7

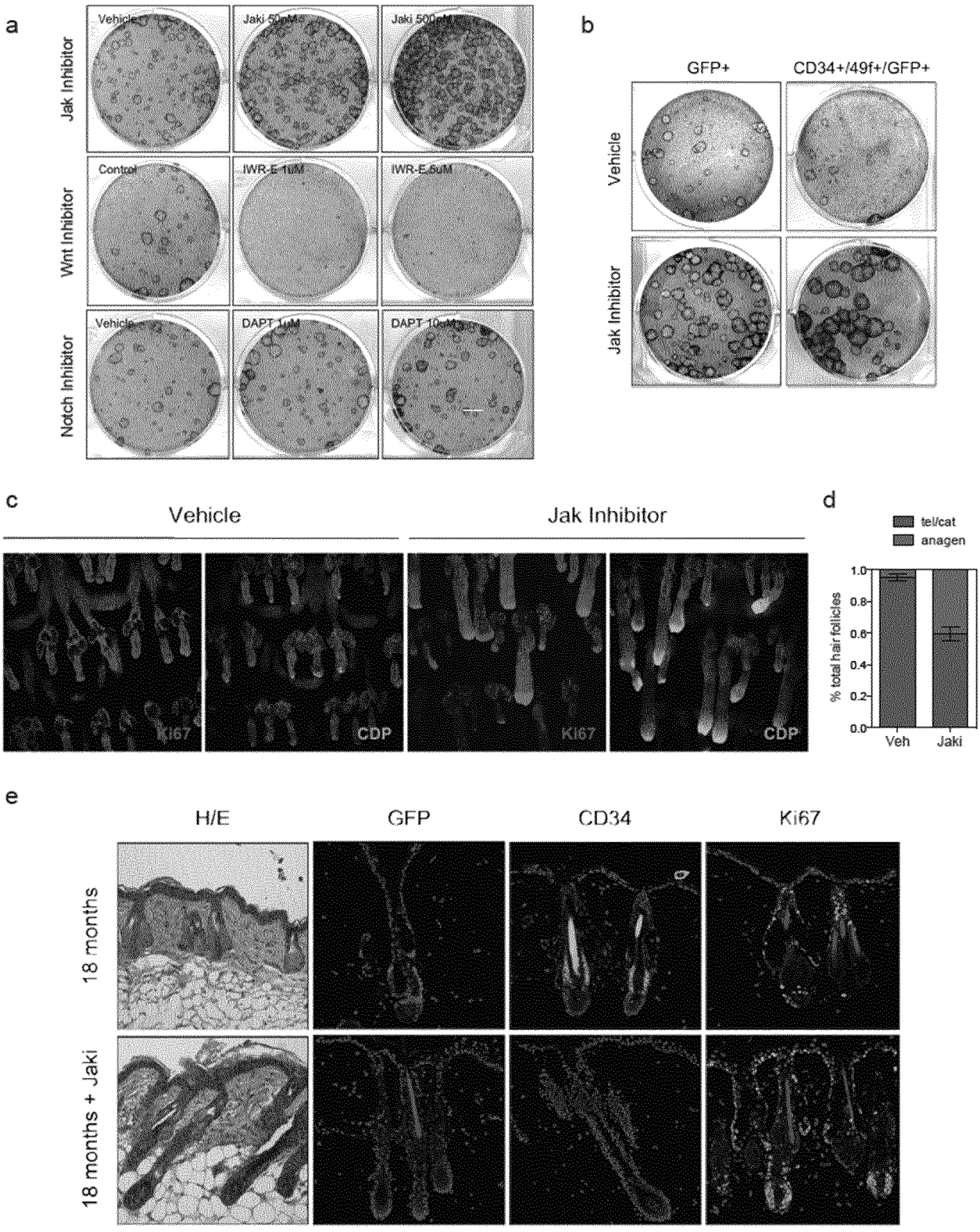

a
Jak Inhibitor
Wnt Inhibitor
Notch Inhibitor
Vehicle
Jaki 50nM
Jaki 500nM
Control
IWR-E 1uM
IWR-E 5uM
Vehicle
DAPT 1uM
DAPT 10uM
b
GFP+
CD34+/49f+/GFP+
Vehicle
Jak Inhibitor
c
Vehicle
Jak Inhibitor
KI67
CDP
KI67
CDP
d
tel/cat
anagen
% total hair follicles
1.0
0.8
0.6
0.4
0.2
0.0
Veh
Jaki
e
H/E
GFP
CD34
Ki67
18 months
18 months + Jaki

Figure 8

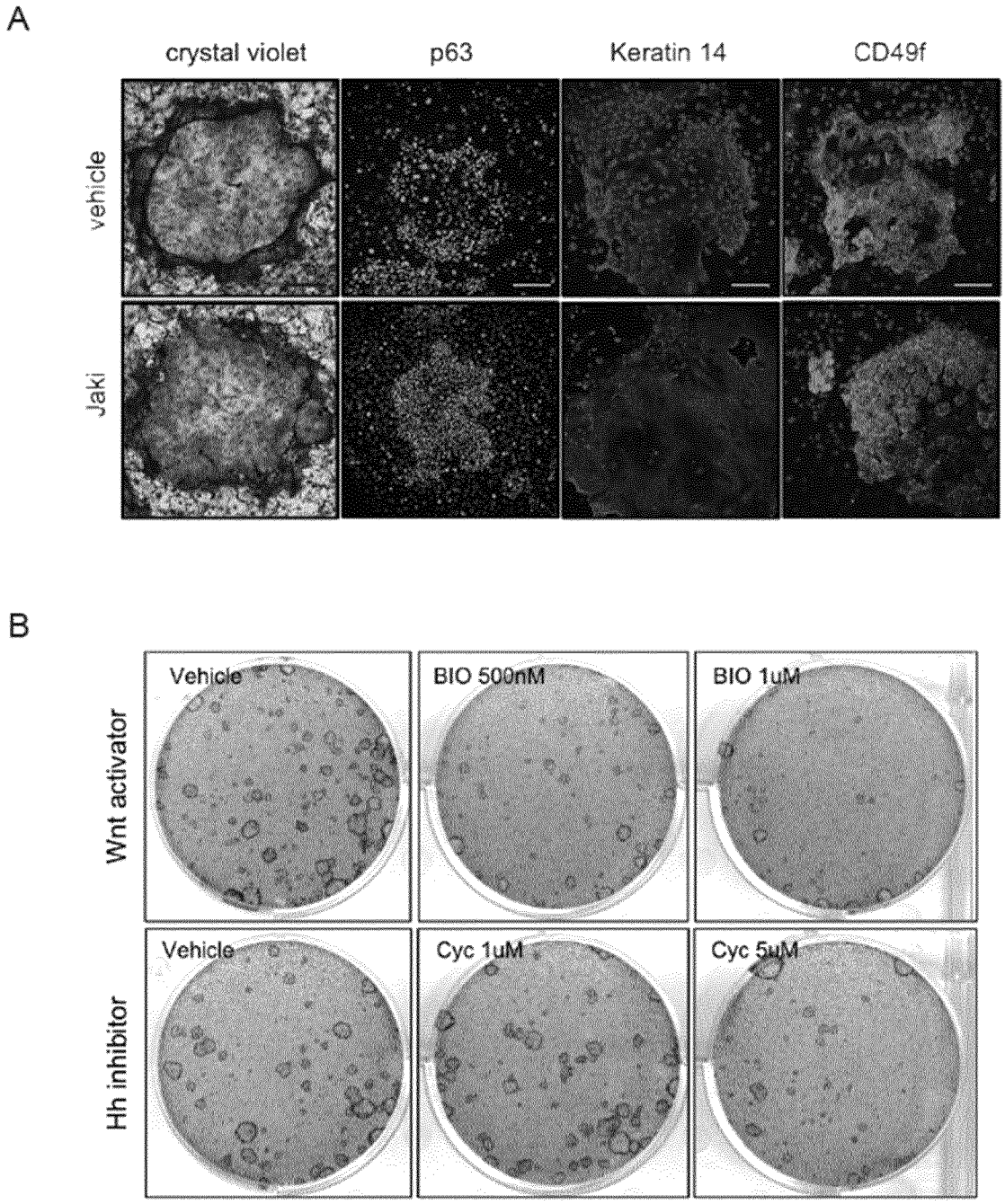

A
crystal violet
p63
Keratin 14
CD49f
vehicle
Jaki
B
Wnt activator
Vehicle
BIO 500nM
BIO 1uM
Hh inhibitor
Vehicle
Cyc 1uM
Cyc 5uM

Figure 9

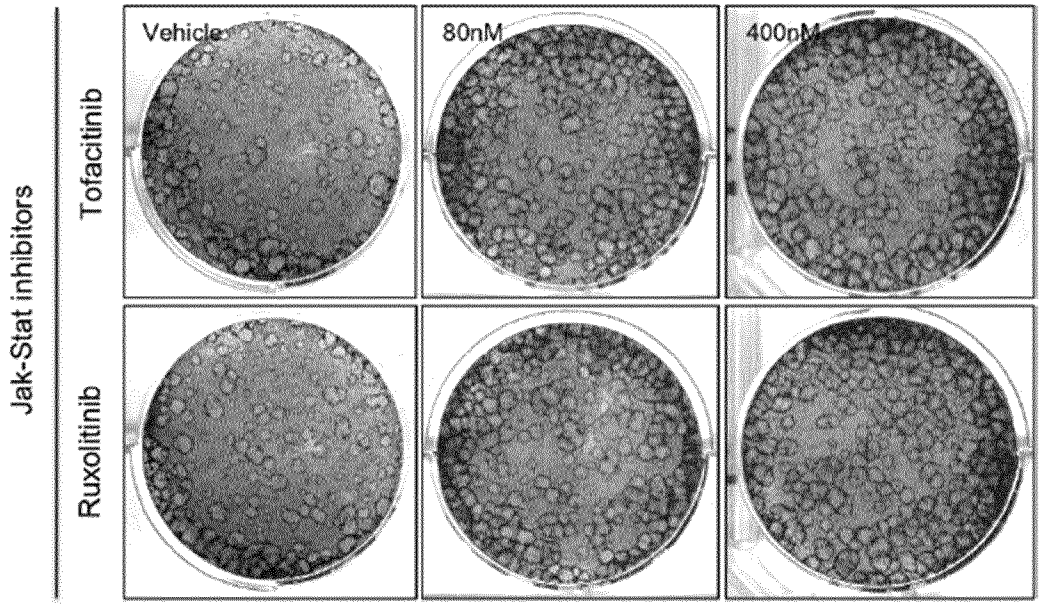
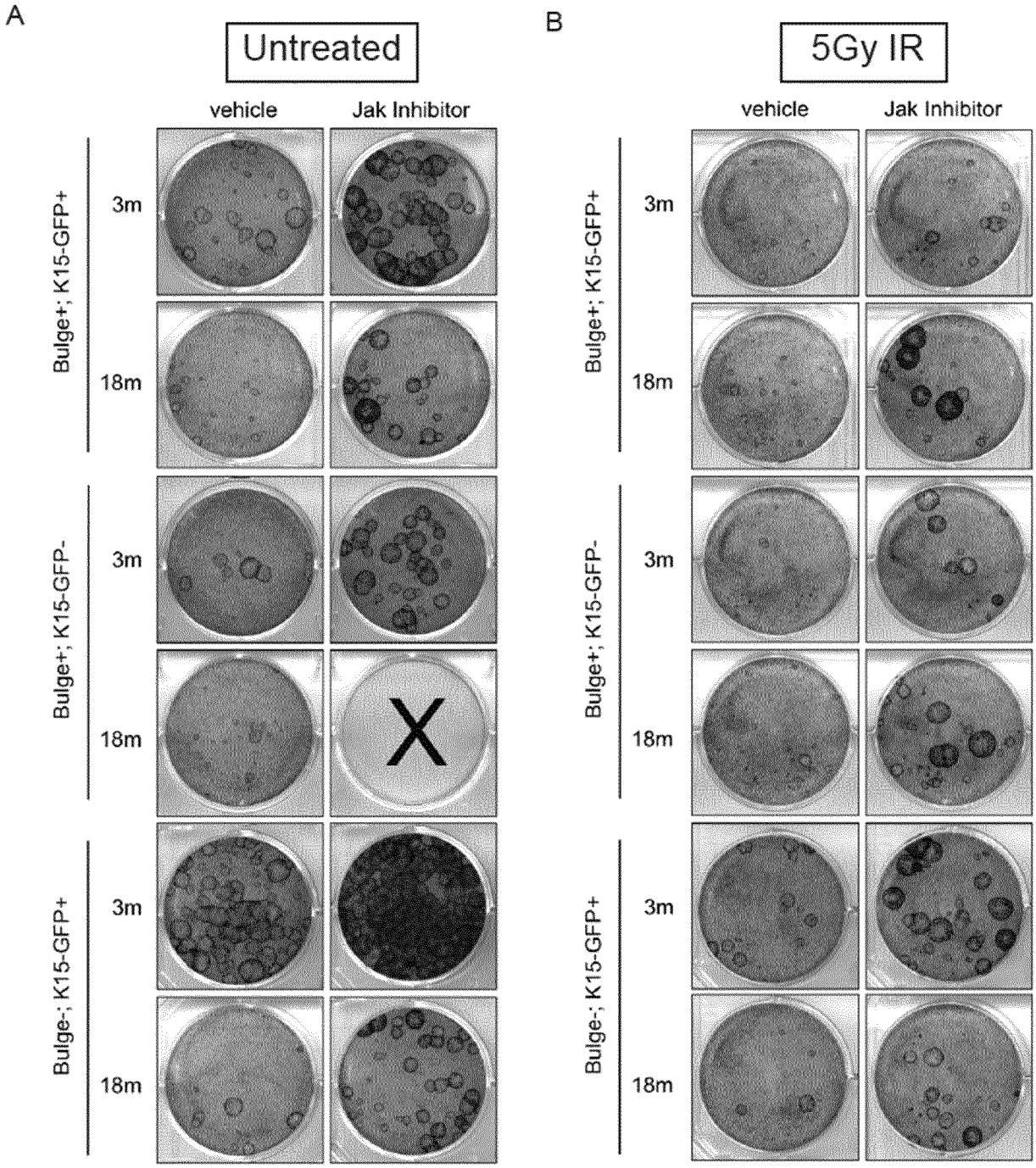

Jak-Stat inhibitors
Tofacitinib
Ruxolitinib
Vehicle
80nM
400nM

Figure 10


A
Untreated
vehicle
Jak Inhibitor
Bulge+; K15-GFP+
3m
18m
Bulge+; K15-GFP-
3m
18m
X
Bulge-; K15-GFP+
3m
18m
B
5Gy IR
vehicle
Jak Inhibitor
Bulge+; K15-GFP+
3m
18m
Bulge+; K15-GFP-
3m
18m
Bulge-; K15-GFP+
3m
18m

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/065177

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/00 A61K31/7105 A61K39/395
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KEYES W M ET AL: "Proffered Paper: Age-associated Cytokine Signaling Impairs Epidermal Stem Cell Function", EUROPEAN JOURNAL OF CANCER, vol. 48, no. Suppl. 5, 8 July 2012 (2012-07-08), page S8, XP002712230, & 22ND BIENNIAL CONGRESS OF THE EUROPEAN-ASSOCIATION-FOR-CANCER-RESEARCH; BARCELONA, SPAIN; JULY 07 -10, 2012 abstract ----- -/--	1,3,7,9, 11,12,14

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2013	16/09/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Wiame, Ilse

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JABBARI A ET AL: "Targeting of JAK3 prevents onset of murine alopecia areata", JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 132, no. Suppl. 1, May 2012 (2012-05) , page S104, XP002712231, & 75TH ANNUAL MEETING OF THE SOCIETY-FOR-INVESTIGATIVE-DERMATOLOGY; RALEIGH, NC, USA; MAY 09 -12, 2012 abstract ----- DAI Z ET AL: "Treatment with Ruxolitinib, an orally bioavailable JAK1/2 inhibitor, prevents the onset of alopecia areata in C3H/HeJ mice", May 2012 (2012-05), JOURNAL OF INVESTIGATIVE DERMATOLOGY, VOL. 132, NR. SUPPL. 1, PAGE(S) S106, 75TH ANNUAL MEETING OF THE SOCIETY-FOR-INVESTIGATIVE-DERMATOLOGY; RALEIGH, NC, USA; MAY 09 -12, 2012, XP002712232, ISSN: 0022-202X(print) abstract ----- WO 2012/061537 A2 (UNIV COLUMBIA [US]; CHRISTIANO ANGELA M [US]; CLYNES RAPHAEL [US]) 10 May 2012 (2012-05-10) paragraphs [0051], [0233], [0290]; claims 1,3-13 ----- SHISHODIA SHISHIR ET AL: "Modulation of transcription factors by curcumin", ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY; THE MOLECULAR TARGETS AND THERAPEUTIC USES OF CURCUMIN IN HEALTH AND DISEASE, SPRINGER, US, vol. 595, 1 January 2007 (2007-01-01), pages 127-148, XP008157947, ISSN: 0065-2598, DOI: 10.1007/978-0-387-46401-5_4 [retrieved on 2007-08-06] abstract page 136, paragraph 1 - paragraph 2 ----- JABBARI A ET AL: "Reversal of longstanding alopecia areata in C3H/HeJ mice using topical JAK inhibitors", JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 133, no. 5, May 2013 (2013-05), page 1395, XP002712233, & 7TH WORLD CONGRESS FOR HAIR RESEARCH; EDINBURGH, UK; MAY 04 -06, 2013 abstract ----- -/--	1-6,9, 11,13 1-3,5,6, 9,11,13 1-3,5,6, 9-11,13, 15 1,3,7-9, 11,14 1-6, 9-11,13, 15

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	J. DOLES ET AL: "Age-associated inflammation inhibits epidermal stem cell function", GENES & DEVELOPMENT, vol. 26, no. 19, 1 October 2012 (2012-10-01), pages 2144-2153, XP055077569, ISSN: 0890-9369, DOI: 10.1101/gad.192294.112 page 2147, column 2, last paragraph - page 2150, paragraph 1 -----	1-15

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/065177

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [ ] on paper

   [X] in electronic form

   b. (time)

   [X] in the international application as filed

   [ ] together with the international application in electronic form

   [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/065177

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012061537 A2	10-05-2012	EP 2635299 A2	11-09-2013
		WO 2012061537 A2	10-05-2012